Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Department of Soil and Environment

Comparing the Diffusive Gradient in Thin Film (DGT) method with water and ammonium-acetate-lactate for P extraction and extractable P required for maximum cereal yield using long-term field experiments

Cornelia Mesmer

Master's Thesis in Environmental Science
EnvEuro – European Master in Environmental Science

Examensarbeten, Institutionen för mark och miljö, SLU
2017:17

Uppsala 2017

Comparing the Diffusive Gradient in Thin Film (DGT) method with water and ammonium-acetate-lactate for P extraction and extractable P required for maximum cereal yield using long-term field experiments

Cornelia Mesmer

Supervisor: Holger Kirchmann, Department of Soil and Environment, SLU
Assistant supervisors: Walter Wenzel, Institute of Soil Research, University of Natural Resources and Life Sciences, Vienna, Austria & Sokrat Sinaj, Agroscope, Nyon, Switzerland
Examiner: Jon-Petter Gustafsson, Department of Soil and Environment, SLU

Credits: 30 ECTS
Level: Second cycle, A2E
Course title: Independent Project in Environmental Science – Master´s thesis
Course code: EX0431
Programme/Education: EnvEuro – European Master in Environmental Science 120 credits

Place of publication: Uppsala
Year of publication: 2017
Cover picture: DGT devices after deployment, 2017, photo by author
Title of series: Examensarbeten, Institutionen för mark och miljö, SLU
Number of part of series: 2017:17
Online publication: http://stud.epsilon.slu.se

Keywords: fertilization, plant-availability, agriculture, diffusion, phosphorus efficiency

Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences
Department of Soil and Environment

Popular Science Summary

Novel way to give phosphorus fertilizer recommendations

Not the entire phosphorus in soils is available for plants. The outcome of this study offers a new possibility to tell farmers how much phosphorus (P) is available and should thus be fertilized.

Does more phosphorus fertilizer always lead to higher crop yields? No, that's not the case! The next question would thus be 'where do farmers then know from how much phosphorus they should apply on their fields for not wasting fertilizer?'. Before I started my work, I didn't have any idea. However, there is a fascinating way to answer this question and I would like to tell you the quite complex story. Phosphorus is a main plant nutrient and only in its presence, crops can reach their optimum yield if other factors are not limiting. However, not the entire phosphorus in the soil is available for plants. A large share is bound to the bulk soil and cannot be taken up by plants. Also, water is needed to "make the phosphorus flow" and enable phosphorus to be taken up by plant roots. When the plant-available phosphorus is used up and no further phosphorus can be taken up, crop yields stay the same and do not increase further. This used up amount of phosphorus can be related to a certain amount of fertilizer. and thus represents the amount of useful phosphorus fertilizer that helps to increase yields. Beyond this amount, yields do not become higher but excess phosphorus is transported to lakes by surface-runoff. Have you ever seen lakes that were almost entirely covered by green water plants? This so called algae bloom is exactly caused by the enrichment of the water with nutrients such as phosphorus and nitrogen (eutrophication) and not seldom lead to fish death. This is why we should avoid overfertilization by recommending farmers adequate amounts of fertilizer.

Now, how can you determine this threshold of plant-available phosphorus when yields do not increase anymore? This was the core of my work. Small plastic devices containing layered gels imitate the phosphorus uptake by plants. In a first step, soil is smeared on top of the device and the phosphorus transport from the soil through one of the gels can start. Phosphorus accumulates in the other gel and is afterwards measured with a photometer. In a second step, results of the plant available phosphorus are related to yields and the value when the highest or optimum yields were achieved represents the threshold. At the end, you get a certain number, let's say 17 kg phosphorus fertilizer per hectare and year, with which the highest yields were achieved. If farmers keep applying this amount of phosphorus fertilizer, yields should stay constant at this maximum level. Of course, one has to measure and check the whole calculation again after some years as soil pH, climate, field management etc. can vary. The great advantage of the method to determine the plant-available phosphorus is that you don't have to send soil samples to laboratories which, due to regulations, often causes problems, particularly between countries. Here, only a solution containing the gel with the bound phosphorus can be sent to virtually any laboratory of the world. To my opinion, this method should be made accessible for the farmers of the world 1. for not wasting fertilizer and 2. for not causing eutrophication of surface waters.

Abstract

Using more phosphorus fertilizer than needed generates unnecessary costs for farmers and can lead to eutrophication of surface waters. Up to now, most country-specific soil tests to determine P plant-availability use chemical extractions that often extract P from non-plant available pools and are not suitable for all soil types. The diffusive gradient in thin film (DGT) method was introduced as a mechanistic surrogate of plant-available phosphorus using water and diffusion in order to quantify P release. In this study, the DGT method was examined and compared with water and ammonium-acetate-lactate extraction. Water extractable P and P-DGT were highly correlated. In order to determine critical thresholds for maximum yields, soil samples from six sites from a long-term field experiment in Sweden and from one site in Switzerland, cultivated with cereals and fertilized with different P rates were used. Piecewise regression and the Mitscherlich model were used to fit extracted P data to relative yields and the piecewise regression provided better fits. Determination of DGT seemed to be superior over the two other P extraction methods to determine critical threshold values for maximum yield but all were useful.

Table of contents

1 Introduction

Among others, phosphorus (P) is an essential plant nutrient determining growth and yields of crop plants. However, phosphorus concentrations in soils differ greatly around the world depending on both natural conditions and anthropogenic input. Globally, 29 % of the world's cropland area reveals P deficits, while 71 % of the cropland area show P surpluses emphasizing low P use efficiency (MacDonald et al. 2011). Due to the growing world population and thus food demand (The World Bank 2016), P fertilizer usage is still increasing (FAO 2015). Although updated numbers reveal considerable amounts of phosphate rock resources and thus do not give any reason for immediate measures to decrease P fertilizer application, the long-term usage of this non-renewable resource and especially the P efficiency in crop production should be reconsidered (Jasinski 2017). Additionally, phosphorus applied to fields is, at the global scale, lost primarily through erosion, runoff and leaching leading to soil degradation and eutrophication of aquatic ecosystems, to mention only the most serious effects of P loss (Schröder et al. 2010). Possible reasons of P over-fertilization in different parts of the world might be (1) the partly low/subsidized P fertilizer price, (2) the missing awareness of farmers of the restricted plant-availability of phosphorus depending on several soil properties and plant-specific strategies in the rhizosphere and 3) the lack of opportunities by farmers to let investigate the P fertilizer amount needed for their fields. A reliable method to predict phosphorus plant-availability is needed that can ultimately serve to recommend farmers the necessary amount of P fertilizer and would thus prevent P over-fertilization and eutrophication.

Measuring the plant-availability of phosphorus usually takes place prior to planting of crops and numerous methods are used for that purpose. In a study comparing various soil-P tests from different countries, Neyroud & Lischer (2003) showed large differences in plant-available P predictions making comparability of test results difficult. Besides that, predictions were reported to be poor when correlating P measurements with relative yield, plant uptake or specific activity, particularly across different soil types (Menzies et al., 2005; Mason et al. 2010, Tandy 2011; Six et al. 2012; Schick et al. 2013). Acidic extractants such as Bray and Mehlich (1984) solubilize Ca-P, Al-P and Fe-P (Nelson et al., 1953) and following competition of anions with P for adsorption sites on the solid phase displaces adsorbed P and hinders re-adsorption of P (Bray & Kurtz 1945; Mehlich, 1984). The very strong acidity of the AL-extract (pH 3.75) used in Sweden hydrolyzes P in insoluble Al-humic-P substances and leads to the dissolution of apatite not being water-soluble (Otabbong et al. 2009). Thus, acidic extractants may extract P from non plant-available pools making them not reliable as a test to determine plant-available P. Furthermore, acidic extractants are most suitable for acidic and neutral soils (Thomas and Peaslee, 1973). The $NaHCO_3$ extractant used in the Olsen soil-P test (Olsen et al., 1954; Colwell 1963) is considered more appropriate for acid and alkaline soils by some authors (Kuo, 1996), while others claim the method overestimate P-availability. This overestimation is due to bicarbonate ions not only releasing P from calcium phosphates but also solubilizing Fe- and Al-phosphates that are not available for plant uptake in some cases (Six et al., 2012). As outlined by Eriksson (2009), extraction force does not only differ due to the extractant and pH but

depends also on soil:solution ratio, as well as the power and duration of shaking the samples. Additionally, soil properties such as pH, clay, carbonate and Fe-, Al-, and Ca-content influence extractable P test results considerably enabling direct comparisons (Schick et al. 2013). Generally, the reliability of chemical extractions seems questionable and their different mechanisms and restricted application to certain soil types make them hardly compatible. While some P tests could be easily converted into others including a variety of soil parameters, others failed to be transformed (Neyroud & Lischer 2003, Schick et al. 2013, Otabbong et al. 2009). According to Neyroud & Lischer (2003), even P-predictions obtained with the same method differed largely due to different laboratory standards. In order to uniform P-availability tests across countries, one common test for extractable P, which is less depend on the strength of extractants and conditions for extraction could be applied.

Diffusive Gradients in Thin Films (DGT) is a more recently developed dynamic sampling and speciation technique to assess P availability in soils. The dynamic DGT method has been shown to be superior to equilibrium-based batch extraction both theoretically and in terms of its predictive power for assessing phytoavailable phosphorus in soil (Degryse et al. 2009). Furthermore, DGT was reported to show widely reliable results without being influenced by carbonate content or anions and, in contrast to conventional extraction techniques, can be applied to acidic, neutral and alkaline soils. Varying pH (3-9) and increasing concentration of anions (chloride, sulfate and nitrate); neither of them showed an effect on the ratio of DGT concentration to P concentration in a solution (Mason et al. 2008).

1.1 Theory and concept of DGT

Originally developed to measure trace elements in natural waters in 1994, DGT was applied to quantify trace element fluxes in sediments and soil in the following years (Davison & Zhang 1994). The simple DGT device consists of a binding gel and is overlain by a hydrogel and membrane. For phosphorus, the use of ferrihydrite gels as a binding agent was proven to be adequately and performed well. When undisturbed moist soil paste is applied to the DGT device, phosphate ions diffusive from the soil solution through the membrane and the hydrogel (diffusive layer) to be bound by the ferrihydrite gel (Davison et al. 2015). As phosphorus is assumed to be delivered to plants mainly through uptake-driven diffusion and desorption from the soil solid phase, DGT, which similarly acts as an infinite sink, can serve as a mechanistic surrogate of bioavailable phosphorus (Knight & Mcgrath 1998).

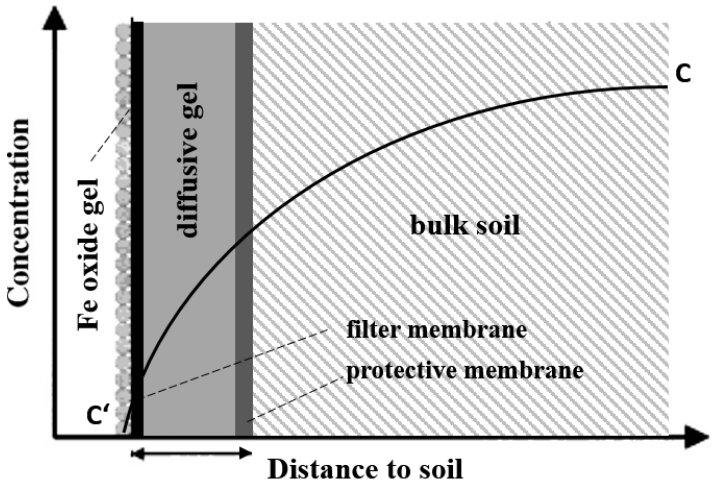


Fig. 1: Schematic depiction of a P concentration gradient in a DGT device at steady state (after Zhang et al., 1998).

Phosphorus plant availability depends on the P speciation in soil. Mineral phosphorus is relatively stable and releases P ions when weathered that quickly precipitate as Al-/Fe- or Ca-phosphates. In the soil solution, phosphorus occurs as a free ion (PO_4^{3-}) or as H_2PO^- and HPO_4^{2-} depending on soil pH. Being the most reactive P form, free ions are relatively fast sorbed to the solid phase, which is described by the solid-liquid distribution coefficient or K_d. When phosphate diffuses into the DGT device, the ion concentration in the soil close to the diffusive layer (C) is lowered inducing a P-desorption and resupply of phosphate ions from the solid phase (Fig. 1). While the resulting P flux can be described as a steady state in waters, the flux from the soil solid phase to the binding layer is larger at the beginning of the DGT deployment time and flattens out till the end of the usual 24 hours of deployment (Davison et al. n.d.). Thus, the directly measured flux of labile species can be understood as the time-averaged concentration cDGT of labile species (free ions or labile complexes) at the interface between the DGT device and solution (Zhang & Davison 1995). Related to P plant uptake, phosphorus accumulated during the DGT deployment is assumed to represent the P concentration available for roots of a plant. The ferrihydrite gel has a capacity of ∼2.5 µg P cm^{-2}, which is equivalent to a cDGT of 450 µgL^{-1} for a 24 h deployment with a standard DGT device at 25°C (Zhang et al. 1998, Menzies et al. 2005). Thus, saturation of the gel may arise under these conditions from cDGT ∼300µgL−1 (Degryse et al. 2009). If cDGT is smaller than the concentration in bulk pore-water, it can be assumed that complexes are present that are not labile during the DGT deployment time. With the ratio of cDGT to total solution concentration giving information about dissociation kinetics of complexes, DGT serves as a dynamic P-speciation tool (Scally et al. 2003).

1.2 Yield response

In order to assess the predictability of plant-available phosphorus using DGT, plant yields can be correlated with DGT concentrations presupposing that other essential nutrients are not limiting crop yields. Responsiveness of plant yield to applied phosphorus is often determined by the "critical value" above which relative yield is 90 % (Holford et al. 1985). Since plant uptake and DGT fluxes were reported to correlate, critical DGT concentrations (representing critical values) can be calculated indicating nutrient deficiency in soils. Figure 2 depicts critical concentrations for four different plants, showing plant-species specific concentrations. Factors influencing critical concentrations are mainly maximal growth rate and root properties (Degryse et al. 2009).

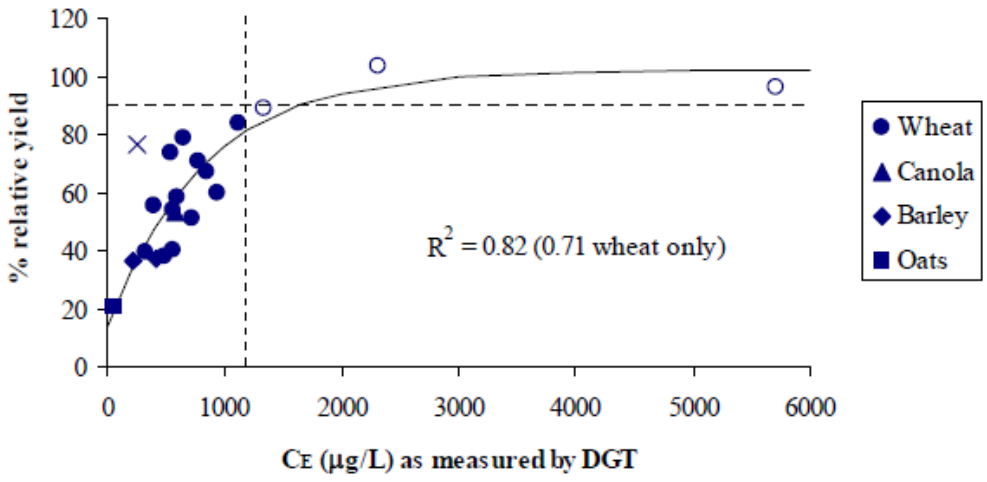


Fig. 2: Critical DGT concentration of four different crops (Mason, et al. 2008).

Considering the sound mechanistic basis of DGT for simulating plant uptake, and its independence of calcium carbonate content and pH fluctuations, this method holds not only promise as a scientific tool but may also serve as a universal standard soil test for assessing P fertilizer requirements in agricultural soils. On top of that, soil samples stay undisturbed transferring representative volumes of samples to authorized soil and planting testing laboratories, which is often limited by law can be avoided with DGT. Using the DGT technique, sampling of the soil and loading the DGT can be done on-site and only the very small, loaded DGT gels need to be mailed. As this can be done without sanitary permission, large numbers of samplings could be performed even in remote regions, while the measurement can be carried out in virtually any laboratory of the globe. This offers the opportunity for enhancing the efficiency of measuring extractable P in particular in less developed regions in e.g. Asia, Africa, where phosphorus inefficiency is highly pronounced and the availability of sound soil and plant testing is limited (Portch & Stauffer 2005).

1.3 Performance of DGT compared to other methods and hypotheses

In the last two decades, numerous studies have been published demonstrating convincingly the suitability of DGT to assess available soil P for plant uptake (Table 1). Data show that if phosphorus uptake is diffusion limited, DGT appears to accurately determine yield response to P fertilizers and more specifically for wheat (Mason et al. 2008; Mason et al. 2010; Speirs et al. 2013), maize (Six et al. 2012; Six et al. 2014) and tomato (Menzies et al. 2005). However, contradicting results of the DGT P assessment were reported for rice and pasture (Six et al. 2012; Burkitt et al. 2016). Focusing on the effective P uptake by plants, Tandy et al. (2011) as well as Heidari et al. (2016) found good correlations between plant-available P predictions and shoot- or root-P.

Table 1: Studies investigating the performance of DGT and conventional extractions to assess P availability in soil.

Performance of extractions and DGT	Climate	Soil type	P rate	Source
DGT, Olsen: good correlation with specific activity (SA)[1] of plant shoot and extracts	Kenya	6 % clay, pH 4.6 Low P sorption capacity	Low and high P rate	(Six, Smolders, et al. 2012)
Big difference of SA in plant shoots and extracts for Olsen, but not for DGT	"	65 % clay, pH 4.2 Strong P sorption capacity	Low and high P rate	(Six et al. 2012)
DGT explained 74 % of variation in wheat response compared to Colwell and resin; critical P threshold (C_{DGT}) = 255 µg L^{-1} for early dry matter and 66 µg L^{-1} for grain.	Southern Australia	35 field trails, different soils	Different P rates, min. 2	(Mason et al. 2010)
49.6 % of the variation in CAL P was explained by C_{DGT}. Separation of data based on relative carbonate content (R^2 = 0.762 calcareous; R^2 = 0.852 non calcareous) and further separation based on sites (R^2 = 0.886, R^2 = 0.954, R^2 = 0.887, 0.871) no correlation of extractable P with plant tissue concentrations CAL and DGT: no significant relationship between extractable P and relative yield	Austria	4 different sites with differing $CaCO_3$ contents, Chernozem and Luvisol	- 0, 75, 150, 300 kg P_2O_5 ha^{-1} a^{-1}. - no P addition, single superphosphate (SSP) and 'basic slag' at P application amounts of 44 kg ha^{-1} a^{-1} and 175 kg ha^{-1} a^{-1}.	(Hill 2016)
Correlation of relative yield to DGT P concentrations (R^2 = 0.74) compared to Olsen (R^2 = 0.60) and AEM (R^2 = 0.62); critical concentration (80 % of yield) = 51µg P L^{-1} (maize)	Kenya	Two Ferralsols, 65 und 78 % clay In a greenhouse, maize was grown on them	0 to 52 kg P ha^{-1}	(Six et al. 2014)
Correlation of DGT and CaCl2 with relative yield (R^2 = 0.84 and 0.69) in comparison to Olsen, Colwell, Bray-1, Mehlich-3, ammonium oxalate and resin extractions (R^2 < 0.53). For rice the other way around Critical DGT concentration (80 % growth): 73 µg P L^{-1} (maize)	Madagascar, Vietnam, Kenya	9 different soils with 6-91 % clay; maize and rice were grown in greenhouses	Various rates	(Six, Smolders, et al. 2012)
Correlation of Colwell and Bray with relative tomato yield poorly, Resin R^2 = 0.83, DGT R^2 = 0.93	Australia	24 different soils, clay 13-72 %	natural high P status/high P fertilizer rate	(Menzies et al. 2005)
correlation with plant yield (mostly wheat) and Colwell: not significant; resin R^2 = 0.43; DGT R^2 = 0.82	Southern Australia	20 different sites	Variety of P responses	(Mason, Mcneill, et al. 2008)
P DGT and Olsen-P solution correlation R = 0.98. Correlation P uptake by corn shoots and Olsen-P/DGT R^2 = 0.79 and R^2 = 0.77. Correlation P uptake by corn roots and Olsen-P/DGT R^2 = 0.83 and R^2 = 0.88.	Aserbaidschan	10 different calcareous soils	Olsen P from 3.1 to 24.6 P mg kg^{-1}	(Heidari et al. 2016)
CE of DGT and barley leaf tissue P concentrations: logarithmic relationship that accounted for 72 % of the variance compared to Olsen P and soil solution P (centrifuged and filtered)	Denmark and Southern Sweden	14 different agricultural soils, clay 3.6 – 18.5 %	Deficient and sufficient P	(Tandy et al. 2011)

[1] In isotopic dilution techniques, the quantification of specific activity (SA) of P in plants grown on a soil labeled fwheawith radioactive P indicates labile P pool (Six, Pypers, et al. 2012).

With little doubt, the DGT technique provides a robust alternative to conventional P tests giving precise information about the nutrient status of soils or necessary amounts of P-fertilizer respectively (Zhang et al. 2013). The diffusive gradients in thin films technique has been calibrated with the help of some of the mentioned long-term field experiments and was commercialized as a soil P test in Australia (Australian Perry Agricultural Laboratory, Magill, Australia). Up to now, most studies that related P predictions to yield were performed on Australian or tropical soils. In order to establish common reference values for soil P availability of certain crops or for recommendations of P fertilizer application, more soil types from different climate zones should be included. Thus, the aim of this study was to investigate DGT performance compared to the two common soil tests for extractable P that differ in their extracting mechanism: AL-P using anion exchange and acid dissolution and water extractable P using the mechanism of desorption enhancement (Jordan et al. 2012). The study comprised soil samples from two P fertilization long-term experiments, one conducted in Switzerland and the other carried out on six different sites in central and south Sweden as to obtain results across soils with different mineralogical and chemical composition, textures, and climatic conditions.

Hypotheses were:

1. The DGT method is a mechanistic surrogate of plant uptake and superior to equilibrium-type chemical extraction procedures to estimate potential cereal yields if P availability is diffusion limited and only P is the limiting nutrient.
2. As DGT and soil solution concentrations have been reported to co-vary strongly, extracted P results of DGT and water extraction are expected to correlate well.
3. All three P-testing methods can be used to determine the critical value of extractable P required for maximum cereal yield.

1.4 Restrictions of DGT predicting P-availability for plants

Although the DGT method takes into account several processes such as diffusion, slow desorption and contribution of complexes that are not considered in conventional chemical P extractions, calculated P plant uptake fluxes were shown to differ from measured DGT fluxes (Degryse et al. 2009). This might be due to several reasons. First, the approximate saturation of the soil during the DGT deployment compared to the generally lower soil water content during plant growth results in larger diffusion fluxes for DGT than under field conditions (Hooda et al. 1999). Second, flux differences can be caused by different deployment times. DGT is usually deployed for 24 hours and P uptake of plants occurs during several weeks (Syers et al. 2008). Third, root properties and its geometry influence P uptake considerably. Quantitatively more and finer roots and root hairs can exploit large portions of the soil as they have a higher specific surface area and mobilize more P than desorbed through soil water only. Moreover, mycorrhizal fungi help to extend the plants' root system and may contribute to P uptake even in phosphorus-rich agricultural soils several microorganisms increase P solubilization (Berruti 2015; Hamel & Strullu 2006). Finally, roots themselves can change the roots' surrounding soil properties. Soil pH is influenced by the ratio of cation/anion excess by plants, whereby the form of nitrogen

plays the most important role. Organic acids exudated by roots may also enhance P availability by altering the speciation of mineral elements (Mikkelsen 2013). DGT, as well as all other P-tests, does not account for the mentioned biotic factors and thus only represents abiotic factors influencing P-availability for plants.

2 Material and Methods

2.1 Sites and samples

In this study, soil samples from one site in Switzerland and from six agricultural fields in Sweden were used.

2.1.1 Swiss experiment

The Swiss samples were provided by and taken at Agroscope in Changins, Switzerland (altitude 432 m) on a field trial with 525 g clay kg^{-1} and 163 g sand kg^{-1} in the top 20 cm soil layer. Mean annual temperature was 9.2 °C and precipitation 999 mm. Since 1971, rapeseed, winter wheat, maize, and winter wheat alternated in a four-year-rotation. The soil was plowed to a depth of 20-25 cm from 1971 to 1985 and only tilled to 12-15 cm with a harrow after 1985. Soil samples (0-20 cm) were taken in October 2012 after the maize harvest. At least eight cores with a diameter of 2.5-3.0 cm were taken randomly from each plot. Plant residues were removed from the soil and individual core samples were mixed to form one composite soil sample per plot. Soil samples were air-dried and sieved to > 2 mm prior to analysis (Gallet et al., 2003).

2.1.2 Experimental design

Each year, the soil was fertilized with five treatments: (A) 0P0K: no P and K applied; (B) 0PK: no P applied and K applied in quantities equivalent to crop uptake; (C) P0K: P applied in quantities equivalent to crop uptake and no K applied; (D) PK: P and K applied in quantities equivalent to crop uptake; (E) PK > exp.: P and K applied in quantities equivalent to crop uptake with an additional fertilization of 26.2 kg P ha^{-1} and 166 kg K ha^{-1}. Prior to plowing or disking, all three crops were fertilized with P as triple superphosphate [$Ca(H_2PO_4)_2$] and K as salt of potash (KCl). All treatments were set in a randomized complete block design and replicated fourth times. Plot size was 15 × 8 m with a 1-m separation between plots. Nitrogen was applied as ammonium nitrate (NH_4NO_3) at the same rate in all fertilization treatments according to the Swiss fertilization guidelines for each crop species (Sinaj et al., 2009). As the no-effect of K fertilization on plant available P was confirmed by the authors, the treatments are referred to (a) and (b): 0P, (c) and (d): P applied in quantities equivalent to crop uptake and (e): P and K applied in quantities equivalent to crop uptake with an additional fertilization of 26.2 kg P ha^{-1} (Gallet et al., 2003).

2.1.3 Swedish experiments

Soil samples from Sweden and respective soil property data were provided by Gunnar Börjesson (2017) and the Plant Nutrition section at the Department of Soil and Environment, Agricultural University of Sweden in Uppsala (SLU) and originate from long-term fertilization experiments of four different sites in southern and two sites in central Sweden (Table 2). The four southern sites are Fjärdingslöv, Orup, Örja and Ekebo and are situated in the county of Skåne. These sites have been cultivated for more than 100 years before the experiment started in 1957 (Börjesson, 2017). The two experiments in central Sweden at Kungsängen and Fors were launched in 1963. The altitude of all sites is between 4 m and 75 m above sea level. The

Table 2: Soil properties of sites and climate data (temperature and precipitation means for 1961–1990 registered at the nearest meteorological station; Alexandersson et al., 1991).

Site	Mean an. temperature (°C)	Mean an. precipitation (mm)	Soil class[1]	Soil texture[1]	Clay %[2]
Changins	9.2	999	Gleyic Cambisol[3]	clay[3]	52[3]
Fjärdingslöv	8.1	590	Haplic Phaeozem	Sandy loam	17
Orup	7.1	777	Haplic Phaeozem	Sandy loam	13
Örja/Borgeby	8.0	569	Eutric Cambisol	Sandy clay loam	15
Ekebo	7.8	683	Eutric Cambisol	Coarse-loamy	14
Kungsängen	5.5	528	Gleyic Cambisol	clay	56
Fors	5.0	635	Calcaric Phaeozem	silty	18

[1] Kirchmann 1991, Kirchmann & Eriksson 1993; Kirchmann et al. 1999.
[2] initial clay content in 0-20 cm depth (Carlgren & Mattsson 2001)
[3] Gallet et al., 2003

cold-temperate and humid climate is similar at all sites with slightly higher winter temperatures in the South compared to the sites in Central Sweden (Carlgren & Mattsson 2001). The soils were classified according to the FAO guidelines (FAO, 1990) by Kirchmann & Eriksson (1993), Kirchmann et al. (1999) (southern experiments) and Kirchmann (1991) (central experiments). Fjärdingslöv and Örja were described by Ivarsson & Bjarnason 1988 as favorable, mainly due to their parent material and high clay content.

Physical and chemical soil properties of the four main sites are depicted in Table 3. With pH values of 7.8 and 7.0, Fjärdingslöv and Örja represent neutral or slightly alkaline, while Orup and Ekebo are characterized by rather acidic soils. Bulk density in kg dm^{-1} increases in the order Ekebo < Orup < Fjärdingslöv < Örja and the clay content is the highest at Örja and Ekebo. Moreover, maximum water held at field capacity as well as organic carbon content is highest at Orup and Ekebo. Only at Fjärdingslöv $CaCO_3$ and Vermiculites constitutes a noteworthy share (0.84 % and 3.15 %). Total P decreases in the order Fjärdingslöv > Ekebo > Orup > Örja.

Table 3: Physical and chemical properties of the topsoil at the four main sites. Fjärdingslöv and Örja are considered as favorable sites. Data from Kirchmann (1991), Kirchmann & Eriksson (1993) and Kirchmann et al. (1999).

Site	pH	Clay (%)	Bulk density (kg dm^{-1})	FC (mm)	$CaCO_3$ (%)	Org. C (%)	Vermi-culites (%)	Smec-tite (%)	Tot. P (%)[1]
Fjärdingslöv	7.8	13.6	1.7	269	0.84	1.28	8	0	0.69[3]
Orup	5.3	12	1.53	335	0	2.44	5	0	0.19[2]
Örja	7.0	23	1.76	287	0.06	1.4	2	0	0.16[2]
Ekebo	6.6	17.8	1.46	321	0	2.38	4	27	0.57[3]

[1]Total P was determined by dissolution of soil in 7M HNO_3 followed by ICP determination.
[2]Total P in (%) of the humus containing fine earth fraction of the soils.
[3]Total P in (%) of the soils.

2.1.4 Experimental design

All selected sites are without livestock and four rates of PK fertilizer (A, B, C, D) were included. In order to exclude nutrient deficiency, only treatments with the highest of four N fertilizer levels were used. The treatments were set in randomized blocks in a split-split plot design with PK on subplots and N on sub-subplots. However, the split-split plot design was incomplete at Kungsängen and Fors. Each year, only one crop was grown in the rotation. Crop rotations are shown in Table 4. Before 1988, the crop rotation of the central Swedish experiments was slightly different with crops 2, 3 and 4 being spring wheat, fallow and oilseeds. Crop residues were incorporated in all rotations. The four levels of P and K follow the principle of replacement: None, replacement of PK removal by the crop and two levels of additional P and K (Table 4; Börjesson, 2017). Average data from all Swedish experiments, including four further sites not used in this study, show a yearly P removal of 15 kg ha^{-1} which was replaced with fertilizer accordingly. In this paper, the replacement P fertilized is sometimes referred to as 15 kg ha^{-1} $year^{-1}$, in order to illustrate P rates in absolute numbers.

In the Southern experiments, PK fertilizer was applied before sowing of spring barley and after the winter wheat harvest. At the central Swedish sites, spring barley and the first winter wheat in the crop rotation was fertilized with PK. As P fertilizer, mono superphosphate (9 % P) was used until 1991 in the south and until 1994 in the central experiments. Thereafter, triple superphosphate (20 % P) was applied. Potassium chloride (50 % K) has been used throughout the experiment. Nitrogen was applied yearly in spring to each crop as nitro chalk (28 % N). Further plant nutrients were added if required. During the experimental period, the southern Swedish sites were limed in the following years: In 1981, Fjärdingslöv and Örja were limed with 2 t ha^{-1} CaO and Orup and Ekebo with 1 t ha^{-1}. In 1996, 2 t ha^{-1} CaO were applied at Orup and Ekebo. Orup and Ekebo were limed again in 2013 (Nordkalk Plus 8.3 t ha^{-1}) and 2014 all southern sites were limed (Nordkalk Plus 5.4 t ha^{-1}) (Börjesson, 2017).

Table 4: Crop rotations and fertilizer amounts of Swedish sites (Carlgren & Mattsson 2001) *and the Swiss site (Gallet et al., 2003).*

	Southern sites[1]	Central sites[2]	Changins
Crop rotation	Barley Oilseed Winter wheat Sugar beet	Barley Oats Oilseed Winter wheat Oats Winter wheat	Rapeseed Winter wheat Maize Winter wheat
PK levels (kg ha^{-1} $year^{-1}$)			
A	No PK		0P0K
B	Replacement of PK removed with crops	+15 P and 40 K (south)	0P and replacement of K
C	Replacement	+20 P and 50 K (central)	0K and replacement of P
D	Replacement	+30 P and 80 K (south & central)	Replacement of P and K
E			Replacement of P and K +26.2 kg P ha^{-1} and 166 kg K ha^{-1}
N level (kg ha^{-1} $year^{-1}$)			
	150	125	According to Swiss fertilization guidelines

[1]Southern sites refer to Fjärdingslöv, Orup, Örja and Ekebo

[2]Central sites refer to Kungsängen and Fors

In order to achieve comparable soil-P results, soil samples were used only from years in which winter wheat was grown. Wheat varieties during this time period were the following: from 1962 Slacke, from 1974 Starke II, from 1990 Folke, from 1994 Kosack and from 2014 Julius. Topsoil samples (0-20 cm) were taken in autumn after the harvest and total biomass of winter wheat was determined (Börjesson, 2017). Sampling years used in this study were 1967, 1979, 1991, 2003 and 2015 and were selected so as to cover a large time range of P recovery or removal respectively. Two field replicates were taken from every plot, but some of them were mixed to a composite sample afterwards. Table 5 gives an overview about used samples in this study. Samples from the two central sites Kungsängen and Fors that fulfill the above-mentioned requirements were only available for the years 1967 and 1979.

Table 5: Overview of soil and yield samples.

Sample Country	Years	Replicates	Treatments	Amount of sites[3]	Soil samples	Grain yield
Sweden	1967	2	4[1]	6	48	48
	1979	2	4[1]	6	48	48
	1991	2	4[1]	4	32	32
	2003	1	4[1]	4	16	16
	2015	1	4[1]	4	16	16
Switzerland	2012	4	5[2]	1	20	20
	Total			7[4]	180	180

[1] Treatments were: (A) no PK; (B) replacement of PK removed with crops; (C) replacement of PK removed with crops +15 P and 40 K (south) or +20 P and 50 K (central); (D) replacement +30 P and 80 K.

[2] Treatments were: (a) 0P0K: no P and K; (b) 0PK: no P applied and K applied in quantities equivalent to crop uptake; (c) P0K: P applied in quantities equivalent to crop uptake and no K applied; (d) PK: P and K applied in quantities equivalent to crop uptake; (e) PK>exp.: P and K applied in quantities equivalent to crop uptake with additional fertilization of 26.2 kg P ha^{-1} and 166 kg K ha^{-1}.

[3] 4 sites refers to Fjärdingslöv, Orup, Örja and Ekebo and 6 sites refers to Fjärdingslöv, Orup, Örja, Ekebo, Kungsängen and Fors

2.2 Extractions

2.2.1 DGT gel preparation and assembling

The basic DGT setup consists of a 0.4 mm thick ferrihydrite-containing hydrogel (binding layer), overlain by a 0.8 mm thick pure hydrogel layer (diffusive layer) and a protective membrane (Fig. 3 and 4). During the entire process of gel preparation and assembling of DGTs, only acid-washed vials and tools and HQ water (18.2 MΩ cm water provided by a Genpure water system 08.2205, ThermoFisher) were used. The diffusive gels were manufactured according to the procedure of Zhang & Davison (1995) consisting of a gel solution of 15 % by volume acrylamide solution (40 %) and 0.3 % crosslinker (DGT Research Ltd). Ammonium persulphate solution (APS) was freshly prepared by dissolving 0.1 g ammonium persulphate in 1 g of HQ water (high-quality water). For 10 ml gel solution, 70 µl of APS and 25 µl of N, N, N'N'-tetramethylethylenediamine (TEMED) solution are added and mixed well. The solution was carefully pipetted in the gap between two glass plates formed by 0.8 mm thick plastic spacers and allowed to set at 44°C for about one hour. Subsequently, up to four gels were washed in ~1 liter of HQ water and the water changed for further 3 times during 24 hours hydration. After washing, the gel was stored refrigerated in 0.03 M $NaNO_3$. To prepare ferrihydrite gels, gels of 0.4 mm using 0.4 mm thick spacers were prepared in the same fashion as the diffusive gels, followed by the precipitation of ferrihydrite according to Santner et al. (2010). Further, 2.70 g of $FeCl_3 \cdot 6H_2O$ (Analar grade reagent, VWR BDH Prolabo, Poole, U.K.) were dissolved in 40 ml of HQ water and up to three gels diffusive gel strips were put into the solution and topped up to 100 g gravimetrically. The final concentration of the solution

was 0.1 mol $FeCl_3$ L^{-1}. The gels were soaked for at least two hours to ensure even distribution of the Fe concentration on the gel surface. Hereafter, each gel was transferred to 100 ml 0.05 M 2-(N-morpholino)-ethanesulfonic acid (Analar grade reagent, VWR BDH Prolabo) that had been buffered with 1 M NaOH to pH 6.7. The gel was stirred gently with a tweezer for about one minute, in order to obtain a homogeneous precipitation (Fig. 5 top left). After another 30 minutes on the plate shaker, gels were washed with ~1 liter of HQ water. The water was changed for further 3 times with at least 2 hours in between to remove excess reagents. Then, gels were conserved in 0.03 M $NaNO_3$ for at least 24 hours and could be used up to 33 days when stored in a refrigerator. Both, diffusive and binding gels were punched to discs of 2.5 cm diameter and could be assembled according to Zhang & Davison (1995). The piston-like plastic housing obtained from DGT Research Ltd (Lancaster, UK) comprises a backing cylinder and a front cap with a 2.0 cm diameter window (Fig. 4). Gels were placed on the cylinder in the order ferrihydrite gel, filter membrane (Sigma: Nuclepore Track-Etch Membrane, Whatman Filtration products, No. 110606, 25 mm, 0,2 µm), diffusive gel and a protective membrane (VWR: Sartorius Biolab Products, No. 15406-25-N, Polyethersulfone membrane filter, 0.45 µm) on top (Fig. 3). Carefully, the front cap was pressed down entirely until a good seal was formed at the filter surface (Zhang & Davison 1995). Moistening the top filter with HQ water, DGT assemblies were stored upright in moistened tight plastic bags in the fridge.

Fig. 3: DGT device with different layers.

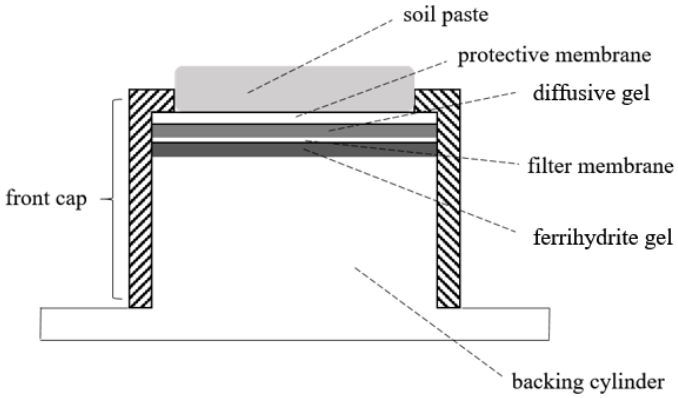
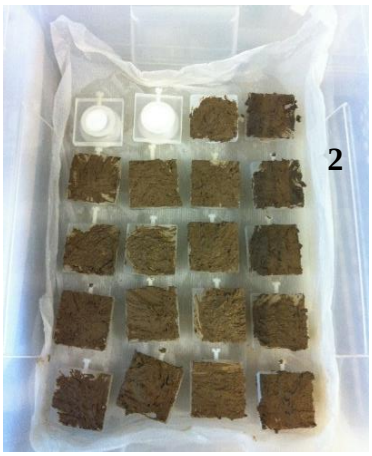


Fig. 4: Schematic cross-section of DGT device (after Zhang & Davison, 1995).

2.2.2 DGT deployment

For the soil paste, maximum water holding capacity (MWC) was determined by saturating one soil sample per site and year to 100 % with water by visual inspection (Blum et al., 1996). For P measurements, the soil was moistened with water to 85 % of the MWC, as (Hooda et al. 1999) suggested to deploy soil with a moisture of 80 % to 100 % of the MWC. Vials were sealed with parafilm and incubated at 20°C for 24 hours, allowing an equilibrium of the dissolved and sorbed fractions of soil phosphorus. In order to avoid drying of the soil during the DGT deployment, wet tissue paper was placed into the incubator a few hours in advance. DGTs were taken out of the fridge at least one hour before the DGT deployment and the soil paste was carefully smeared on the DGT samplers and incubated for another 24 h at 20°C. Wet tissue paper was placed on the bottom of the box with DGTs to create a moist environment during the DGT deployment (Fig. 5 top right). Two replicates per soil samples were carried out. Further two blank DGT's without soil paste, as well as two reference soils with known DGT-P concentrations for quality control, were measured for every ~30 DGTs. The following day, the soil paste was removed from the DGT sampler and remaining soil particles were carefully wiped (Fig. 5 bottom left). Subsequently, both membranes and the diffusive gel were discarded, while the Fe-oxide gel was rinsed with HQ water in case of adhering soil particles (Zhang & Davison 1995). For the phosphate elution, ferrihydrite gel discs were placed in 5 ml 0.25 M H_2SO_4 and put on a plate shaker for ≥ two hours. After another 24 hours, the gels were measured using the modified molybdate blue colorimetric method according to Zhang et al. (1998). Thereafter, the 0.25 M H_2SO_4 solution, in which the gels were stored, was mixed with ascorbic acid and a reducing agent. The mixture was prepared in 1.5 ml cuvettes and compared with the blue-violet color of P standards (0.02-0.5 mg P L^{-1} in H_2SO_4) (Fig. 5 bottom right). If sample solutions were darker than standards and therefore out of the measurable range, samples were adequately diluted with H_2SO_4. P was measured at the wavelength 881 λ using a Hitachi U2000 photometer (Tokyo, JP). If P-concentration within the range of 0-0.25 mg P L^{-1} showed standard deviations exceeding 15 % and within 0.25-0.5 mg P L^{-1} 7 %, respectively, DGT measurements were repeated with a third replicate to achieve more accurate results.



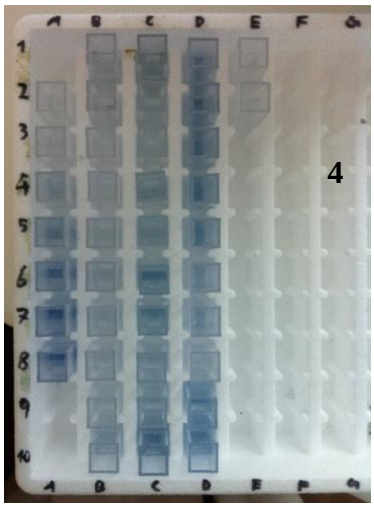


Fig. 5: Different steps of DGT method. 1. ferrihydrite precipitation. 2. Saturated soil paste smeared on DGT devices with two blanks. 3. Removal of soil paste from DGT devices. 4. molybdate blue colorimentry for photometer measurement.

For the calculation of the time-averaged phosphate flux, Fick's law of diffusion can be used (equation 1).

$$C_{DGT} = \frac{\mathrm{M}\Delta\mathrm{g}}{DAt} \qquad (1)$$

Here, M is the mass of P bound by the ferrihydrite gel, Δg is the thickness of the diffusion layer, D is the P diffusion coefficient in the diffusion layer (provided by DGT Research Ltd.), A is the sampling area and t is the sampling time (Zhang et al. 1998). M can be calculated with the concentration of P in the eluate (CE) referring to the volume of the gel and the added H_2SO_4 (equation 2).

$$M = Ce\ (V_{gel} + V_{acid}) \qquad (2)$$

2.2.3 Water extraction

Water extraction for the Swedish samples was performed according to the method used for the Changins sample (Demaria et al. 2005). To 2.5 g of each soil sample, 25 ml of HQ water was added, shaken by hand for ~ 1 minute and left standing for 24 hours. Then, the solution was shaken for another 16 hours on a shaker with 20 rotations per minute. Afterwards, the soil extract was first filtered through filter paper and then filtered again with 0.4 µm syringe filters to get a clear extract. The extracts were filled up with 0.25 M H_2SO_4 with a 9:1 extract: H_2SO_4 ratio. For each soil sample, two replicates were carried out. The extract was measured using the photometer as described for the DGT measurement.

2.2.4 P-Olsen and P-AL

P-AL data were provided by the Swedish University of Agriculture in Uppsala (SLU), while P-Olsen data was given from Agroscope for the Changins site.

Ammonium acetate lactate (P-AL) is the standard test for plant available P in Belgium, Hungary, Lithuania, Norway, Slovenia and Sweden (Jordan et al. 2012). The method was developed by Egner et al. (1960) and evolved from the double lactate method (for acidic soils) after having been modified for the assessment of all types of soil. Ammonium serves as a cation in the extractant and allows determining of K, Ca and Mg at the same time (Egner et al. 1960). The low pH (3.75) caused by the addition of acetic acid allows the analysis of soils with up to 20 % carbonate content but has the disadvantage to also dissolve apatite if present (Riehm, 1958).

The Olsen-P test was originally developed for calcareous soils and is still the most common method to determine extractable P for alkaline soils. Basically, Olsen's method (Olsen et al. 1954) solubilizes phosphate in calcareous soils by replacing phosphate by bicarbonate, carbonate and hydroxide ions (Barrow & Shaw 1976). The high pH of $NaHCO_3$ solution leads to a decline of the activity of soil solution Ca^{2+} by precipitation as calcium carbonate. This results in enhanced desorption of P from Ca^{2+} in calcareous or from Fe^{3+} and Al^{3+} in acid soils (Demetz & Insam 1999).

In order to compare the data of the two methods, the regression model of Otabbong et al. (2009) to convert P-Olsen in P-AL values and vice versa was used. For the conversion, the clay content in percent and the pH of the respective soil is needed. The regression models (see equation 1 and 2) were developed by testing 82 Swedish top-soil samples including the six sites used in the present study. Otabbong et al. (2009) found correlations of predicted and measured P-AL and P-Olsen of 0.95 and 0.94.

P-AL predicted

$$= \left(-19.767 + 1.546\sqrt{P_{Olsen}} - 0.467\sqrt{clay\%} + 3.276pH\right)^2 \quad (1)$$

P-Olsen predicted

$$= (12.678 + 0.599\sqrt{P_{AL}} + 0.232\sqrt{clay\%} - 1.985pH)^2 \quad (2)$$

After converting P-Olsen into P-AL data using equation 1, recalculation from calculated P-AL into P-Olsen using equation 2 showed a correlation of R^2 = 0.999 between predicted and measured P-Olsen. Thus, calculated P-AL values were used for further calculations in this work.

2.3 Yield data evaluation and statistical analysis

2.3.1 Representativeness of soil samples

Sample sizes varied between the sites of this study. The four Southern sites of the Swedish experiment Fjärdingslöv, Orup, Örja and Ekebo represent a moderate sample size with four different P rates over five years. In contrast, samples from only the first two years 1967 and 1979 were available for the sites Kungsängen and Fors so that these two sites were excluded in some calculations or graphs (mostly for graphs over time). Similarly, Swiss samples represent a small sample size with three different P rates of one site and one year.

2.3.2 Swedish experiments

Relative crop yields of the Swedish sites were determined as the percentage of the maximum crop yield per site and year. By calculating relative yields both site and climate effects are normalized. In order to test whether the relative yield significantly increased with P application, an analysis of variance (ANOVA) was performed using the R program (R 386 3.4.1). Results of the ANOVA indicated differences of relative yields between treatments; hence the non-parametric pairwise Wilcoxon rank sum test using the Bonferroni method was conducted. Two different methods described in the following were used to depict the relationship between extracted P and relative yield. In this way, critical P threshold values required for maximum yield were obtained.

2.3.3 Data fit to Mitscherlich curve

In order to make the results of the present study comparable with the similar study of Mason et al. (2010) who also examined the power of the DGT method to estimate wheat response under field conditions, the Mitscherlich model was applied. Due to my missing software, Mason (2017) conducted the following steps. A Mitscherlich curve between yield and rate of P for every site and year was fitted for P-DGT and P-AL in the form

$$y = \text{yo} + a\,(1 - e^{-bx}) \quad (3)$$

where y_o = yield of control (0 P) and y_o + a = the maximum yield reached with the highest P application (45 P) calculated with the model. To determine the response at each site per year, the relative yield (%) was calculated with the following equation

$$relative\ yield\ (\%) = \frac{yield\ (control)}{yield\ (maximum)} * 100 \quad (4)$$

where yield (control) is the obtained yield from the control plots (yo) and the yield (maximum) is the maximum yield calculated from Eq. 3 (y_o + a).

Relative yield per site, year and treatment was calculated by dividing the respective actual grain yield by the calculated maximum yield. Relative yields at sites in years without clear response were calculated differently: 1) if sites were not responsive, the calculated maximum yield in Eq. 4 was substituted by the mean of all other P rates except the control, 2) if the response curve seemed to be linear, the calculated maximum yield in Eq. 4 was substituted by the observed maximum yield, and 3) if the relationships between P rate and obtained yield were very poorly (e.g. decreasing yields with increasing P rate), sites of particular years were excluded for soil test correlations.

In order to examine the fit of the Mitscherlich model to the relationship between relative yield and extractable soil P values, a site response prediction method was applied. Hereby, critical P-DGT thresholds (relative yield = 90 %) were determined for the years 1967; 1967 and 1979; 1967 to 1991; 2003 and 2015 and over all years and were compared with those thresholds of the piecewise curve fit. In a second step, it was checked, if P-DGT values for relative yields (determined per site, year and treatment) below 90 % were below the critical 90 % P-DGT threshold of the respective years. If yes, the response was correctly predicted; if not, the response was incorrectly predicted. The more correct the response predictions, the better is the model performance. Results are given in percentage of correct predictions.

2.3.4 Data fit to piecewise linear regression

Due to moderate correlations achieved with the Mitscherlich fit, a second model, the piecewise linear regression fit was employed. Plotting two lines for the relationship between extracted P and relative yields, the piecewise regression fit meets the nature of plant yields approaching a threshold beyond that yields do not increase anymore. Thus, the breaking point or knot of the two lines can be identified as the critical threshold where maximum yields were achieved.
In a first step, P-DGT, P-H_2O and P-AL were plotted against relative yields for each site and year using the piecewise curve fit procedure of the software Sigmaplot 12.0. The regression using the following equation fits the data into two lines identifying a breaking point.

$$f = \begin{cases} \dfrac{y_1(T_1 - t) + y_2(t - t_1)}{T_1 - t_1} & , t_1 \le t \le T_1 \\ \dfrac{y_2(t_2 - t) + y_3(t - T_1)}{t_2 - T_1} & , T_1 \le t \le t_2 \end{cases} \qquad (5)$$

Variables of Eq 5 are:
T_1: breaking point along the x-axis
y_1: lowest value of y-axis for line 1
y_2: highest value of y-axis for line 1 and 2
y_3: lowest values of y-axis for line 2
t_1: help variable used for the iteration of T (minimum value)
t_2: help variable for the iteration of T (maximum value)

This breaking point is defined as the achieved maximum relative yield (100 %) and corresponds to a certain DGT- H_2O- or AL-value before the yield flattens out. The respective soil-test value at the yield breaking point represents the critical threshold in order to achieve maximum crop yield. In a second step, the threshold value for a 90 % relative yield was determined. This value was compared with 90 % critical threshold values derived with the same data set but fitted to a Mitscherlich curve. Coefficients of determination for the regression fits were recorded. Regression fits with negative values were excluded. Mean critical thresholds were calculated (1) per site over all years and (2) across sites and years combined. Relative standard deviations of the means across sites were calculated for all soil-P tests in order to examine the methods' performance, i.e., the smaller the relative standard deviation, the more accurate is the soil test.

2.3.5 Treatment and DGT-change

In order to determine to which extent P fertilizer rates lead to change in P-DGT values, linear regression equations for P-DGT across all sites and years were calculated. Subsequently, the four treatments were plotted against the slope of the P-DGT regression, representing P-DGT change per year for different P application rates per year.

3 Results

3.1 Yield response to applied P

Results of the pairwise Wilcoxon rank sum test showed that the relative yields were significantly increased by P application compared to control treatments ($p < 0.01$) with the biggest difference to the P replacement rate with about 10 % (Fig. 6 and Table 6). However, relative yields did not show significant differences between the replacement and the replacement +15 kg P ha^{-1} and +30 kg P ha^{-1} respectively ($p > 0.05$), but relative yields even decreased by up to 5 % the more P was applied. Grain dry matter yields showed a wide range with minimum yields of 2238 kg ha^{-1}, maximum yields of 8500 kg ha^{-1} and mean yields at the sites increase in the order Orup < Ekebo < Fjärdingslöv < Örja (Table 7). Generally, yields dropped in 1979 before increasing until 2015 due to the change to better performing wheat varieties in the 1970s and the following years (Fig. 7). At Fjärdingslöv, Orup and Ekebo, yields in 1991 for the two upper P rates were lower than for the two lower P rates.

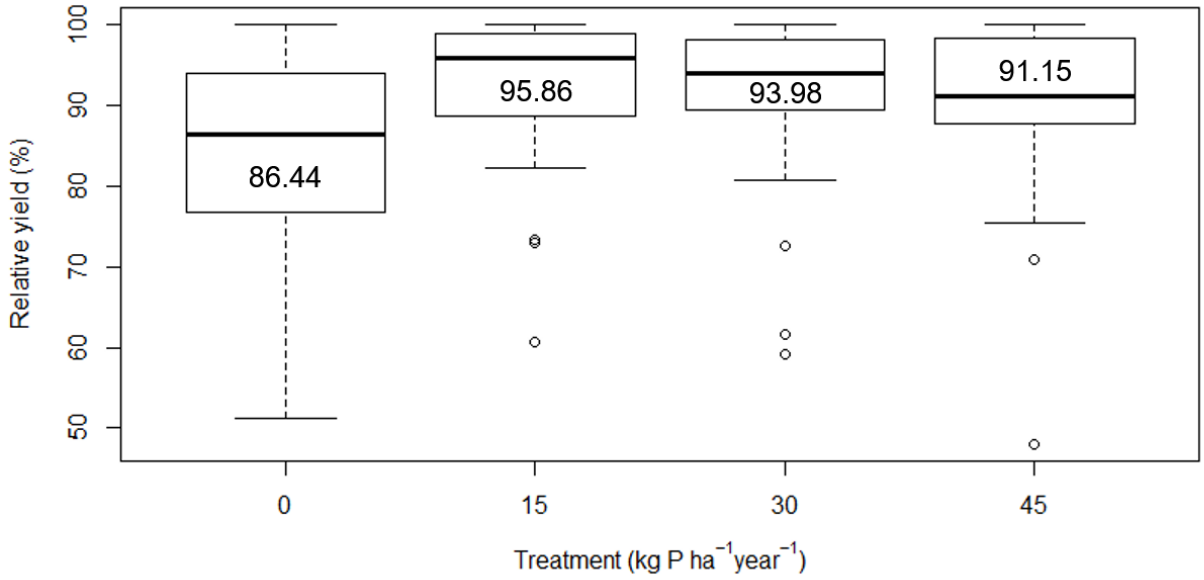


Fig. 6: Differences of relative yield in % depending on the P application. 0 P treatment differs significantly from all other P rates (p<0.01), but no significant differences between replacement (15 P), replacement + 15 kg ha-1 (30 P) and replacement + 30 kg ha-1 (45 P). Median values written in boxes.

Table 6: P-values of pairwise Wilcoxon rank sum test (Bonferroni) for relative yields depending on yearly P rate.

Treatment (kg P ha^{-1} $year^{-1}$)	0	15	30
15	0.003	-	-
30	0.003	1.000	-
45	0.063	1.000	1.000

Table 7: Minimum, maximum and mean of grain yield for different sites over all years.

Site	Grain yield (kg dry matter ha^{-1})		
	Min	Max	Mean
Fjärdingslöv	3563	8453	5382
Orup	3218	5899	4364
Örja	3779	8068	5458
Ekebo	2238	8501	4799

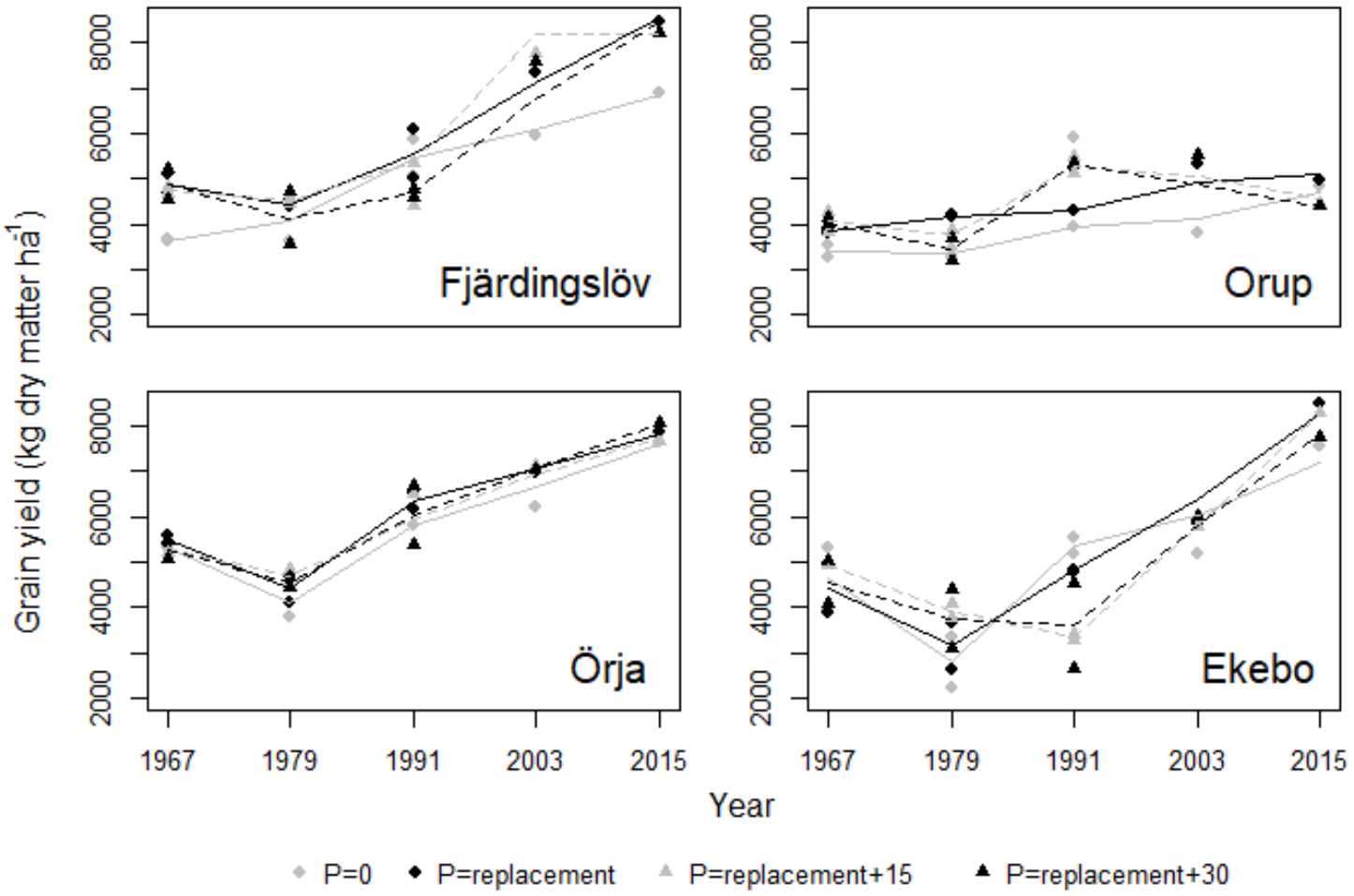


Fig. 7: Grain yield over years of four sites for the four different P rates.

3.1.1 Mitscherlich model

At most sites, wheat response to applied P fertilizer was modeled well by the Mitscherlich function resulting in high coefficients of determination for the regression fit between predicted maximum grain yield and P rate (Table 8). Relative yields per site and year for cases where the P rate – grain yield curve did not follow a Mitscherlich curve were calculated differently explained in the material and methods chapter. Figure 8 shows one curve corresponding and one curve deviating from the typical Mitscherlich curve. For non-responsive sites during certain years, the Mitscherlich curve did not provide a suitable relation between crop yield and P rate. In those cases, relative yield was calculated by substituting the calculated maximum yield with the average yield from all P rates except the control P treatment. Two sites during certain years showed a linear relationship between applied P and yield so that relative yields were calculated using the observed maximum yield. Furthermore, certain years of four sites were excluded from the Mitscherlich model due to very irregular yield responses. It should be marked that most not responsive sites in certain years showed either yield decreases for the highest or the two highest P rates, which is in accordance with the decreasing median relative yields for those treatments (Fig. 6).

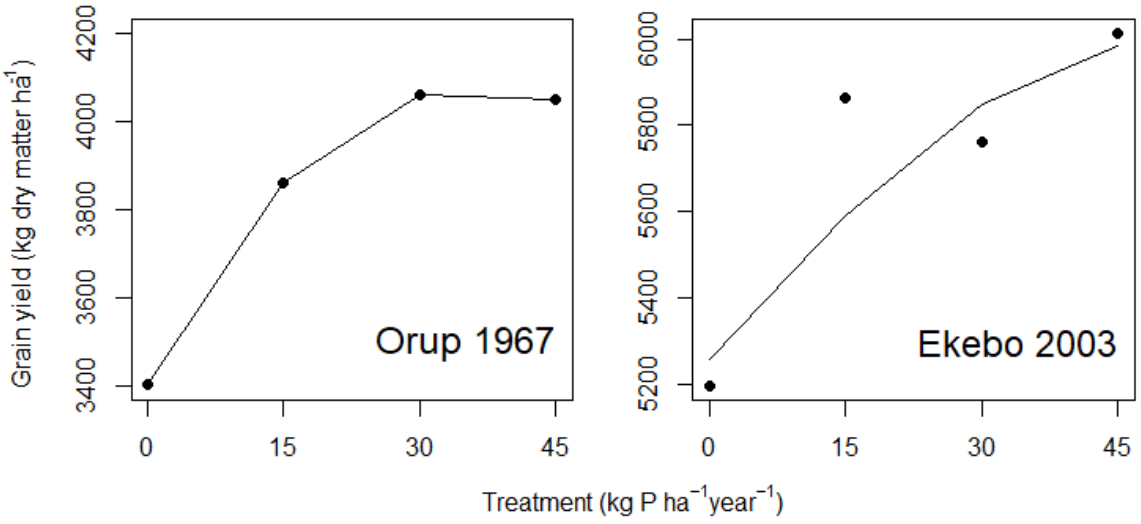


Fig. 8: Relationship between P rate and wheat response (grain yield) at two sites. Response curves seem to conform Mitscherlich model (left) and linear regression (right). Based on such observations, relative yields for the Mitscherlich model were calculated (see methods section).

Table 8: Grain yield and calculated grain yields and relationships with P rate from every site and year. Yield response represented as relative yield (%) using the control and the predicted maximum yield resulting from the Mitscherlich model fit. Sites in certain years that were excluded from the Mitscherlich fit (marked in dark gray) exhibited poor relationships between P rate and yield. Linear and not responsive sites at certain years (marked in light gray) were not used to fit the Mitscherlich curve .

Site	Year	Yield control kg ha^{-1}	Yield max. measured kg ha^{-1}	Yield max. predicted kg ha^{-1}	R^2	Relative yield of control (%)	Yield response
Fjärdingslöv	1967	3646.5	4889.5	4838.8	0.99	75.36	Yes
	1979	4075.5	4505.5	4569.5	1	89.19	Yes
	1991						Excluded
	2003	5952.0	7766.0	7674.3	0.98	77.56	Yes
	2015	6909.0	8453.0	8291.0	0.97	83.33	Yes
Orup	1967	3401.9	4058.5	4093.3	0.99	83.11	Yes
	1979	3355.5	4173.0	3965.8	0.74	84.61	Yes
	1991						Linear
	2003	3791.8	5510.0	5521.5	1	68.67	Yes
	2015						Excluded
Örja	1967						No
	1979	4120.2	4667.5	4630.0	0.90	88.99	Yes
	1991	5996.5	6327.0	6093.8	0.08	98.40	Yes
	2003	6222.6	7127.0	7082.2	0.99	87.86	Yes
	2015						Linear
Ekebo	1967						No
	1979	2759.4	3918.0	4098.7	0.86	67.32	Yes
	1991						Excluded
	2003	5195.6	6014.0	5898.3	0.92	88.09	Yes
	2015						Excluded
Kungsängen	1967	5482.5	5969.0	5946.3	0.99	92.20	Yes
	1979	4671.9	5172.0	5141.6	0.98	90.86	Yes
Fors	1967	5763.5	6090.5	5950.8	0.37	96.85	Yes
	1979	4014.5	5198.0	4965.0	0.80	80.86	Yes

3.2 Soil P extraction methods

3.2.1 Comparison of extracted P analyses

Values for extracted P-DGT ranged between 2.24 and 457.30 µg L^{-1} and mean P-DGT increased in the order Ekebo < Orup < Fjärdingslöv < Örja (Table 9). Water extraction resulted in P values between 0.13 and 17.16 mg P kg^{-1} soil for all sites and increased in the same order (Table 10). The most aggressive extractant AL extracted with P values between 1.1 and 24.6 mg P 100 g^{-1} soil (≙ 110 and 2460 mg P kg^{-1} soil) around 1400 % more P than water. Extracted P-AL increased in the order Orup < Fjärdingslöv < Örja < Ekebo (Table 10). All soils showed a clear positive relationship between extracted P measured with DGT, water extraction and P-AL and applied P (Fig. 9 and 10). Extracted P from different methods stayed relatively constant for the 0 P and replacement P across all years and sites. However, for replacement +15 kg P/ha and replacement +30 kg P/ha, DGT- and water extracted P showed similar extracted P values across the years, while P-AL extracted P differed. In 2003, DGT-P and water extracted P showed a peak for the highest P rates at the sites Fjärdingslöv, Orup and Örja, while P-AL slightly increased at all sites. Only at Ekebo, extracted P values measured by the three methods showed the same increasing trend for the upper two P levels over the years. When comparing extracted P and grain yields over years, extracted P from the three methods showed at all sites a similar increasing trend over the years as grain yields (Fig. 7, 9 and 10). However, water extracted P and P-DGT did not follow the grain yield drop in 1979, but dropped in 1991 at all sites except Ekebo. Furthermore, the relationship of extracted P and grain yield differed, which is shown by the ratio of mean yield and mean extracted P in the last column of Table 9 and 10. Ekebo showed the lowest values of P-DGT and P-water, while yields were the second lowest at this site. This resulted in higher yield/mean extracted P ratios at Ekebo compared to the other sites. In contrast, P-AL was highest at Ekebo and showed a much lower yield/mean P-AL ratio than for the other sites.

Table 9: Minimum, maximum and mean of grain yield and P-DGT for different sites over all years and ratios of mean yield/mean extracted P

Site	Grain yield (kg dry matter ha^{-1})			P-DGT (µg L^{-1})			Mean yield/ mean P-DGT
	Min	Max	Mean	Min	Max	Mean	
Fjärdingslöv	3563	8453	5382	9.71	347.34	95.71	56.23
Orup	3218	5899	4364	2.24	307.93	72.00	60.61
Örja	3779	8068	5458	12.46	457.30	104.90	52.03
Ekebo	2238	8501	4799	4.82	216.35	56.39	85.10

Table 10: Minimum, maximum and mean of P-H_2O and P-AL for different sites and ratios of mean yield/mean extracted P

Site	P-H_2O (mg P kg^{-1} soil)			Mean yield/mean P-H_2O	P-AL (mg P 100 g^{-1} soil			Mean yield/mean P-AL
	Min	Max	Mean		Min	Max	Mean	
Fjärdingslöv	0.14	17.16	3.80	1416	1.30	17.57	6.65	810
Orup	0.13	14.67	2.97	1469	1.10	16.80	4.68	932
Örja	0.26	16.12	4.07	1341	1.90	16.10	6.90	548
Ekebo	0.14	8.19	2.69	1784	2.20	24.60	8.55	262

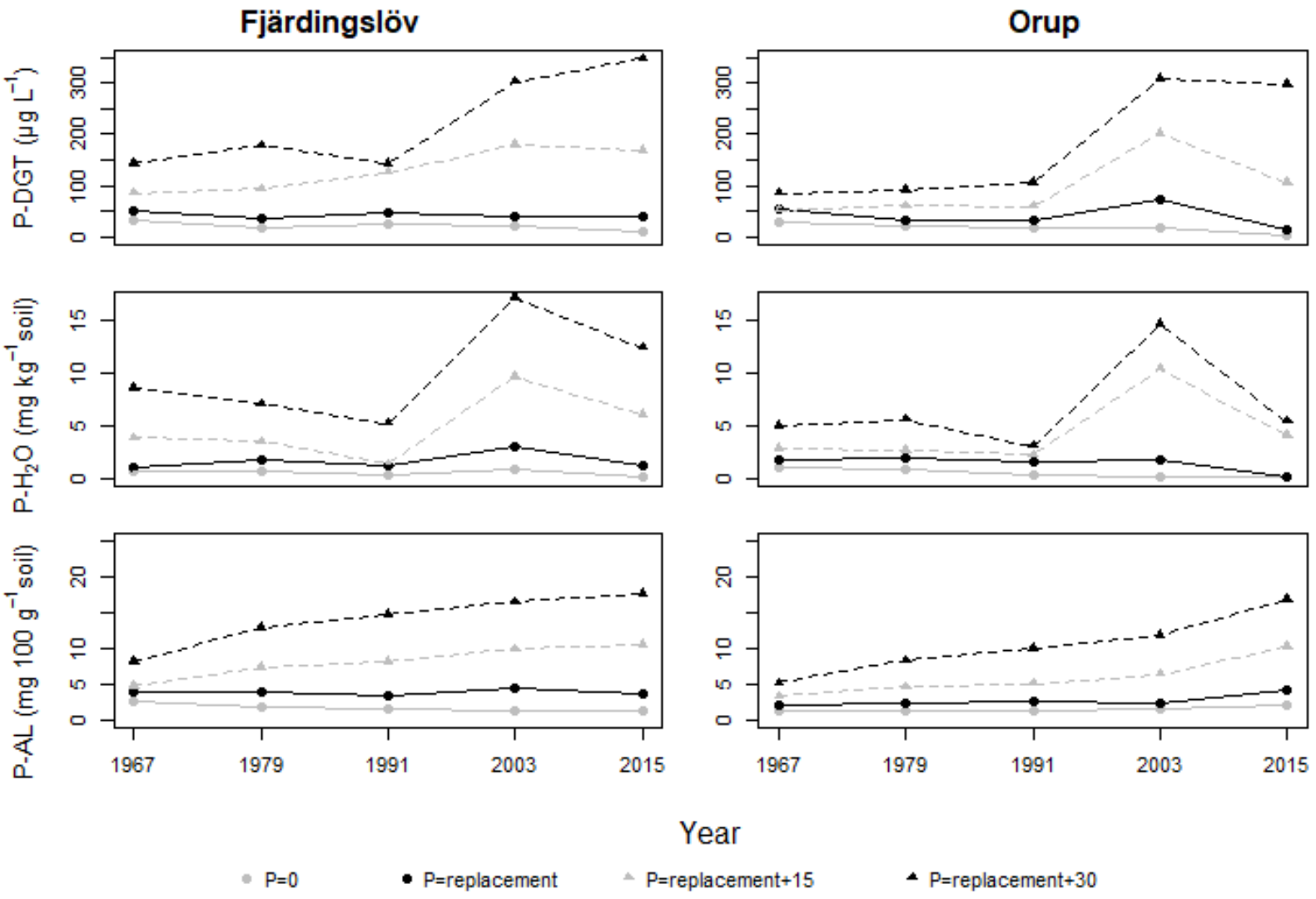


Fig. 9: Extracted P over years measured with the DGT, AL and water extraction method for two sites.

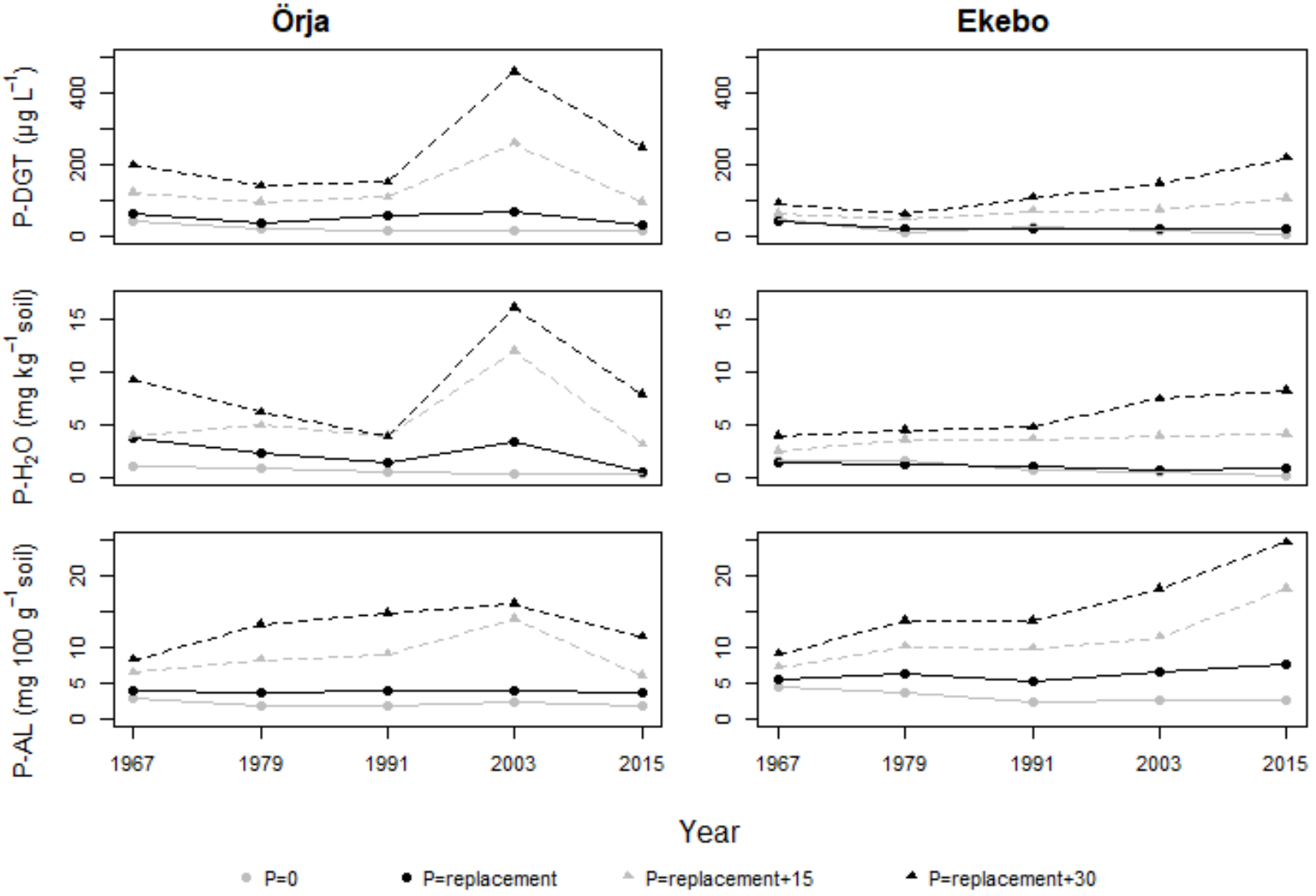


Fig. 10: Extracted P over years measured with the DGT, AL and water extraction method for two sites.

Table 11: Pearson correlation coefficient of extracted P from all years for different P tests.

Relationship between	Fjärdingslöv	Orup	Örja	Ekebo	Kungsängen	Fors	All sites (except Fors)
P-DGT-P-H_2O	0.897	0.858	0.905	0.921	0.671	0.663	0.874
P-DGT-P-AL	0.919	0.836	0.816	0.868	0.846	-0.226	0.750
P-H_2O-P-AL	0.813	0.641	0.730	0.911	0.884	0.015	0.703

Extracted P values exhibited generally high cross-wise correlations. In particular, DGT and water extraction correlated well across all sites except Fors (R = 0.874) and water and P-AL extraction correlated least (R = 0.703) (Table 11). At Fors being a highly calcareous soil, extracted P data derived by different P-methods correlated poorly.

3.2.2 P fertilizer treatments and P-DGT

Data on P-DGT increased with rates of P fertilizer applied at all sites (Fig. 11). The increase of P-DGT was steeper for the sites Fjärdingslöv and Örja than for Orup and Ekebo. Values doubled at all sites from around 25 to up to 50 µg L^{-1} when replacing the yearly removed P (15 kg P ha^{-1} $year^{-1}$) for a period of 48 years except at Ekebo. When applying the replacement P + 15 kg P ha^{-1} $year^{-1}$ over the experimental period, P-DGT increases by more than 100 % up until 140 µg L^{-1} and from there almost doubled for the highest P treatment (replacement + 30 kg P ha^{-1} $year^{-1}$).

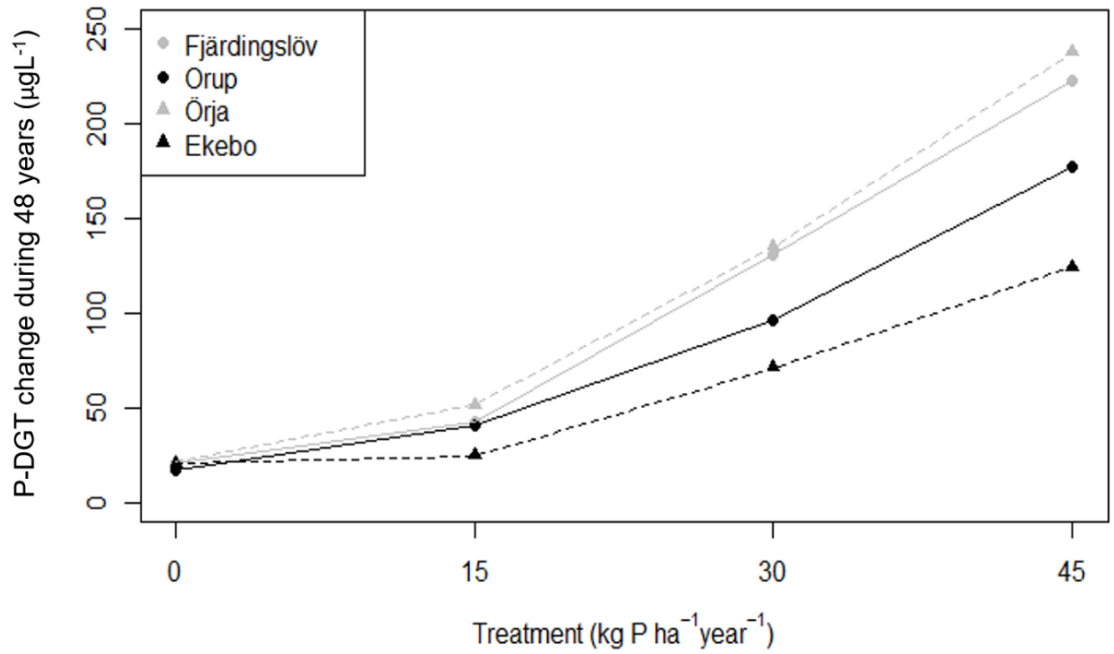


Fig. 11: Relationship between yearly P rate and P-DGT in 2015 (change in P-DGT during the experimental period) at four sites.

3.3 Yield response assessment

3.3.1 Mitscherlich model

Regression fits were performed across sites and including different years, in order to examine yield prediction power of the Mitscherlich model over the years. Using data from the selected sites and years, DGT was relatively effective in predicting yield response to applied P generating moderate to high R^2 for the regression fit for relative yields (Table 12 and Fig. 12). R^2 values were within the range of 0.64 for the year 1967 to 0.36 for all years and generally decreased, the more years were incorporated. Critical P-DGT values obtained for relative yields were between 24.87 and 40.55 µg P L^{-1} with the highest values occurring for 2003 and 2015 (Table 13). Data on P-DGT correctly predicted the yield response to fertilizer P in 60 - 100 % of the cases depending on which years were combined. Again, the prediction power of relative yields using P-DGT decreased the more years that were included. In contrast to P-DGT, P-AL performed weaker with coefficients of determination for the regression fit between predicted maximum grain yield and P rate (R^2 = 0.11 to 0.77) (Table 12 and Fig. 13). However, for the years 2003 and 2015, P-AL produced higher R^2 values than P-DGT. Critical P-AL thresholds for different combinations of years showed values between 1.60 and 2.21 mg P 100 g^{-1} soil. Correct predictions of wheat response to applied P were achieved for 29 - 60 % of the cases for the respective years (Table 12).

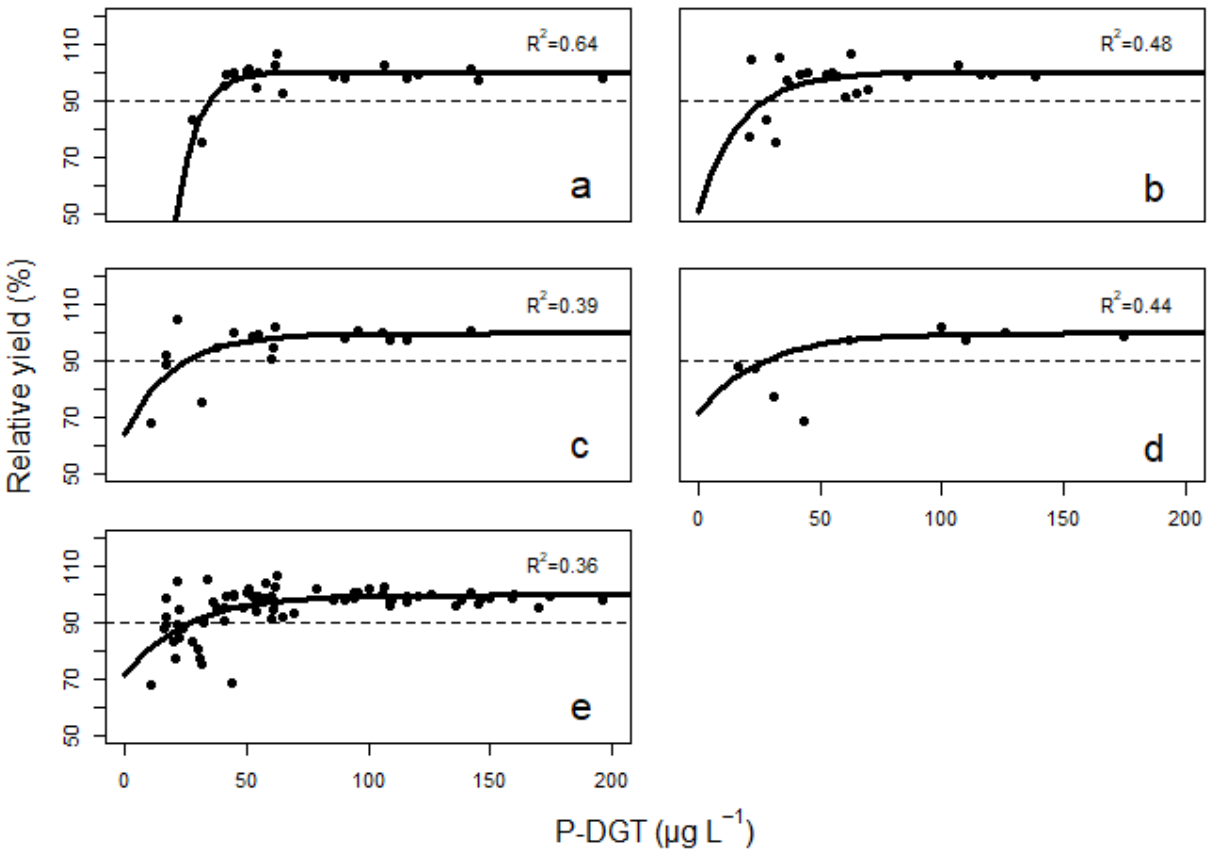


Fig. 12: Relationship of DGT soil test expressed as P-DGT with wheat response (% relative yield) to P applications for the years (a) 1967, (b) 1967 and 1979 (c) 1967 to 1991, (d) 2003 and 2015 and (e) 1967-2015. Solid line represents the fitted Mitscherlich curve and dashed line represents the critical threshold at 90 % relative yield.

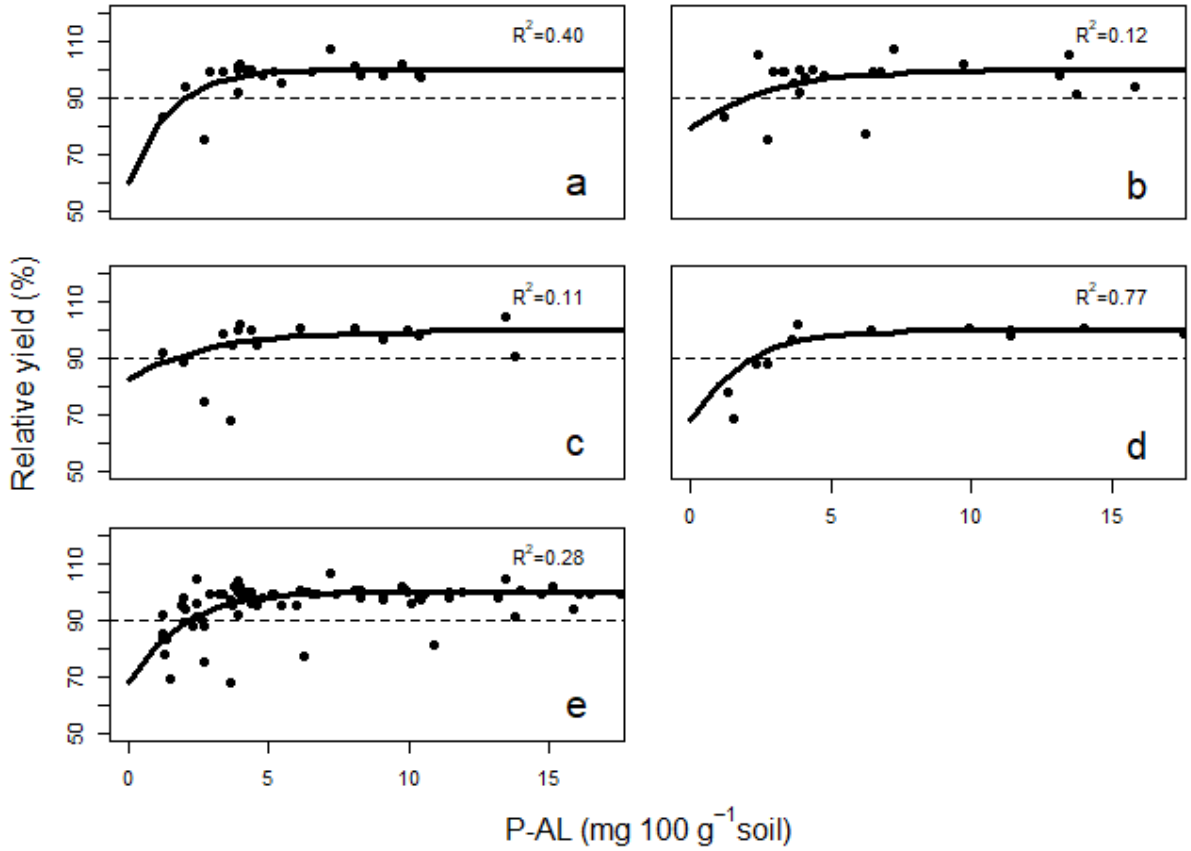


Fig. 13: Relationship of AL soil test expressed as P-AL with wheat response (relative yield) to P applications for the years (a) 1967, (b) 1967 and 1979 (c) 1967 to 1991, (d) 2003 and 2015 and (e) 1967-2015. Solid line represents the fitted Mitscherlich curve and dashed line represents the critical threshold at 90 % relative yield.

Table 12: P-DGT/P-AL, calculated P-DGT/P-AL values, relationship with predicted relative yield and soil test ability to correctly predict the yield response to P-DGT values using the Mitscherlich model. Correct prediction was examined by checking if extracted P for relative yields (from each site, year and treatment) below 90 % were below the critical 90 % threshold of extracted P of the respective years. Percentage reflects the share of correct predictions.

Method	Year	Yo	a	b	R^2 extracted P with max. predicted relative yield	% correct prediction Mitscherlich model
P-DGT (µg L^{-1})	1967	-491.9	591.93	0.12	0.64	100 %
	1967, 1979	50.60	49.40	0.06	0.48	62 %
	1967-1991	64.45	35.55	0.05	0.39	62 %
	2003, 2015	79.08	20.92	0.018	0.44	60 %
	1967-2015	71.30	28.70	0.039	0.36	62 %
P-AL (mg P 100 g^{-1} soil)	1967	59.85	40.15	0.69	0.40	50 %
	1967, 1979	78.86	21.14	0.37	0.12	43 %
	1967-1991	82.76	17.24	0.34	0.11	29 %
	2003, 2015	22.30	77.70	0.93	0.77	60 %
	1967-2015	67.66	32.34	0.56	0.28	50 %

Table 13: 90 % critical yield thresholds of P-DGT and P-AL for different years calculated with the Mitscherlich model.

Method	Year	90 % threshold Mitscherlich model
P-DGT (µg L^{-1})	1967	34.70
	1967-1979	27.35
	1967-1991	24.87
	2003-2015	40.55
	1967-2015	27.04
P-AL (mg P 100 g^{-1} soil)	1967	2.02
	1967-1979	2.01
	1967-1991	1.60
	2003-2015	2.21
	1967-2015	2.11

3.3.2 Piecewise linear regression fit

Curve fits per site and year between extracted P and relative yield showed high coefficients of determination for all soil-P extraction methods, but the highest for DGT (Table 14). Mean P values to obtain 100 % relative yield per site ranged between 63.425 (Ekebo) and 94.873 µg P L^{-1} (Örja) for DGT and 1.832 (Ekebo) and 7.120 µg kg^{-1} soil (Örja) for water extractions respectively, exhibiting similar trends (Table 14). In contrast, P-AL showed with 3.03 mg P 100 g^{-1} soil the lowest critical value at the Örja site and with 7.22 and 12.326 mg P 100 g^{-1} soil the highest values at Ekebo and Fors, respectively. Hence, P-AL critical threshold values deviated from site-dependent critical thresholds for DGT and H_2O.

Table 14: DGT, H_2O and AL critical thresholds per site and year determined at 90 % and 100 % of maximum relative yields determined by piecewise regression of data. Thresholds could not be calculated where numbers are missing. Negative thresholds marked in gray were excluded for the calculation of mean values.

		DGT critical threshold (µg P L^{-1})		R^2	H_2O critical threshold (mg kg^{-1} soil)		R^2	AL critical threshold (mg P 100 g^{-1} soil)		R^2
Site	Year	90 %	100 %		90 %	100 %		90 %	100 %	
Fjärdingslöv	1967	47.480	50.500	0.952	5.160	-3352.390	0.289	2.720	2.700	0.989
	1979	25.970	49.920	1.000	1.220	2.410	1.000	2.940	5.222	1
	1991	31.830	65.370	1.000	-	5.230	0.610	2.737	10.770	0.691
	2003	89.494	114.911	1.000	0.874	0.855	0.778	3.603	5.630	1
	2015	49.136	83.675	0.994	-	-	-	2.219	3.240	0.992
Orup	1967	56.415	25.695	0.659	1.824	2.200	1.000	2.046	2.432	1.000
	1979	26.377	30.289	0.995	-	6.527	0.036	1.886	2.400	0.917
	1991	72.863	65.805	0.792	2.866	1.534	0.770	5.767	6.253	0.776
	2003	115.099	148.113	1.000	1.452	1.972	1.000	2.199	2.522	1.000
	2015	173.208	59.500	0.873	4.430	0.203	0.969	10.534	10.851	0.874
Örja	1967	-	62.000	0.917	-	16.030	0.156	-	3.730	0.987
	1979	36.220	59.368	1.000	2.135	4.114	1.000	3.533	5.763	1.000
	1991	15.216	57.500	0.594	0.366	3.012	1.000	-	-10.901	0.139
	2003	54.379	145.853	0.999	1.049	4.578	1.000	2.721	5.156	1.000
	2015	-	116.769	1.000	-	7.864	0.798	-	11.400	0.765
Ekebo	1967	54.097	62.789	0.935	2.059	2.514	0.936	6.675	7.200	0.489
	1979	41.323	43.163	1.000	-	3.597	0.832	-	-	-
	1991	-	-	-	-	2.465	1.000	-	-	-
	2003	25.507	58.495	1.000	0.450	0.449	0.968	3.341	4.428	0.953
	2015	19.305	89.254	1.000	-	0.137	0.779	3.140	10.034	1.000
Kungsängen	1979	43.959	65.696	1.000	-	-7.061	0.449	2.550	3.966	1
Fors	1979		-34.476	0.000	2.318	3.707	0.173	12.326	14.264	1

Table 15: Extractable P-DGT, -H_2O and -AL thresholds values per site over all years determined for 90 % and 100 % of maximum relative yields by piecewise regression of data. Thresholds could not be calculated where numbers are missing.

Site	DGT critical threshold (µg P L^{-1})		H_2O critical threshold (mg kg^{-1} soil)		AL critical threshold (mg P 100 g^{-1} soil)	
	90 %	100 %	90 %	100 %	90 %	100 %
Fjärdingslöv	48.782	72.875	2.418	2.832	2.844	5.512
Orup	88.792	65.880	2.643	2.487	4.486	4.892
Örja	35.272	94.873	1.183	7.120	3.127	3.030
Ekebo	35.058	63.425	1.255	1.832	4.385	7.221
Kungsängen	43.959	65.696	-	-	2.550	3.966
Fors	-	-	-	2.318	-	12.326

Relative yields showed a steep increase for low DGT values before reaching the breaking point of the two fitted lines and flattening out (Fig. 14 and 15). Typically, relative yields remained constant with increasing concentrations of extracted P after having reached the maximum yield. However, in this study, several cases, such as P-DGT at Orup, Örja and Ekebo (Fig. 14) or P-AL at Fjärdingslöv (Fig. 15) showed decreasing relative yields with increasing extracted P after having reached the critical threshold. Mean critical thresholds and standard errors for 90 % and 100 % relative yield data over all years and split by sites are shown in Figure 16 and 17. Critical thresholds at 90 % were determined in order to be able to compare them with the 90 % critical thresholds calculated by the Mitscherlich model. Derived thresholds for 100 % yield were, on average, 1.5 times (DGT), 1.8 times (H_2O) and 1.2 times (AL) higher than the threshold for 90 % yield (Fig. 16 and 17). The dispersion of data points at each site illustrates the variation of critical thresholds over years. Relative standard deviations were smallest for the 90 % and 100 % critical thresholds of P-DGT (69.64 % and 45.80 %), intermediate for P-AL (70.26 % and 56.07 %) and largest for H_2O (68.72 % and 97.14 %).

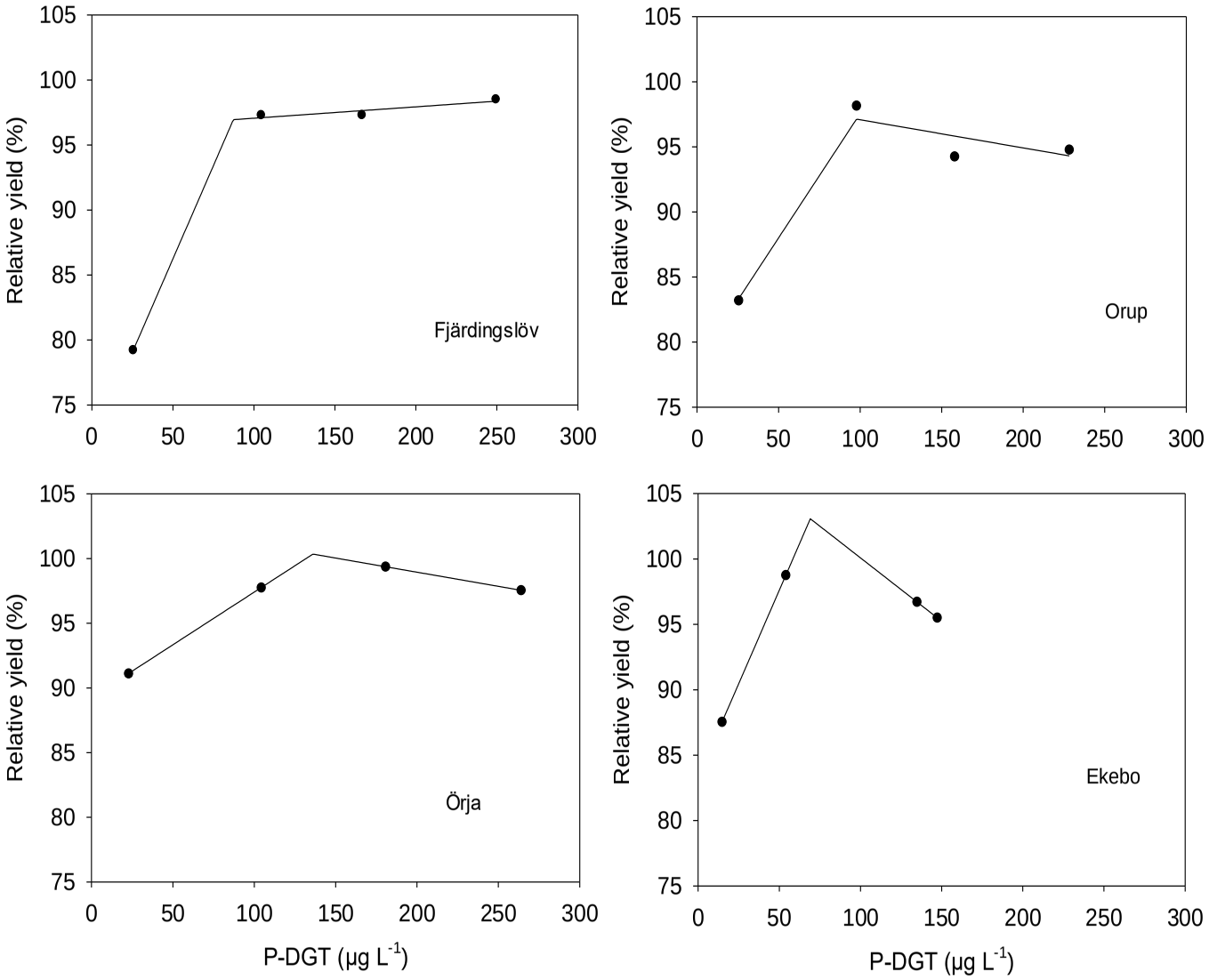


Fig. 14: Relationship of DGT--soil test expressed as P-DGT with wheat response (% Relative yield) at Fjärdingslöv, Orup, Örja and Ekebo. Mean values of 2003 and 2015.

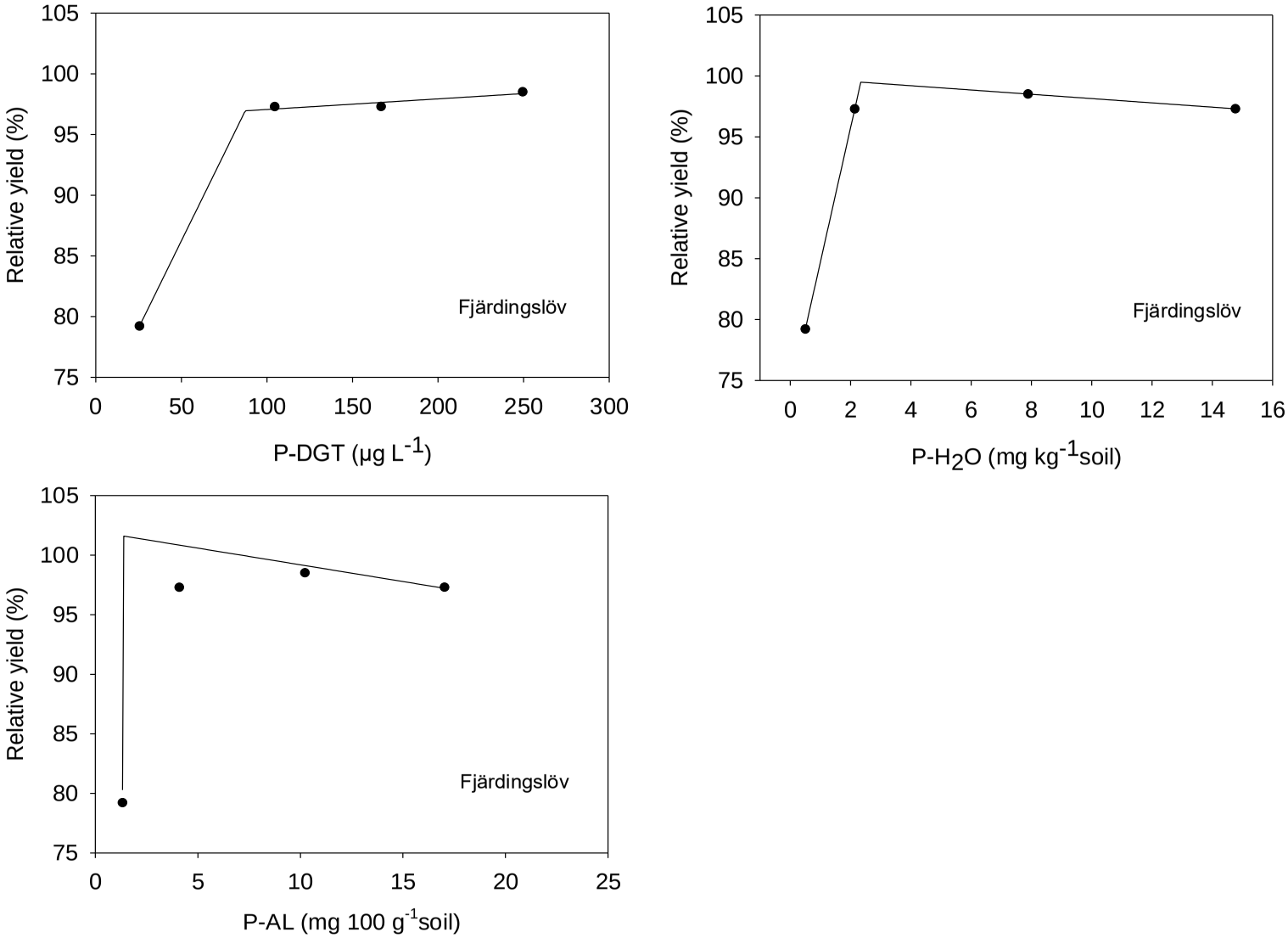


Fig. 15: Relationship of DGT-, H_2O- and AL-soil test expressed as P-DGT, P- H_2O and P-AL with wheat response (% Relative yield) at Fjärdingslöv. Mean values of 2003 and 2015.

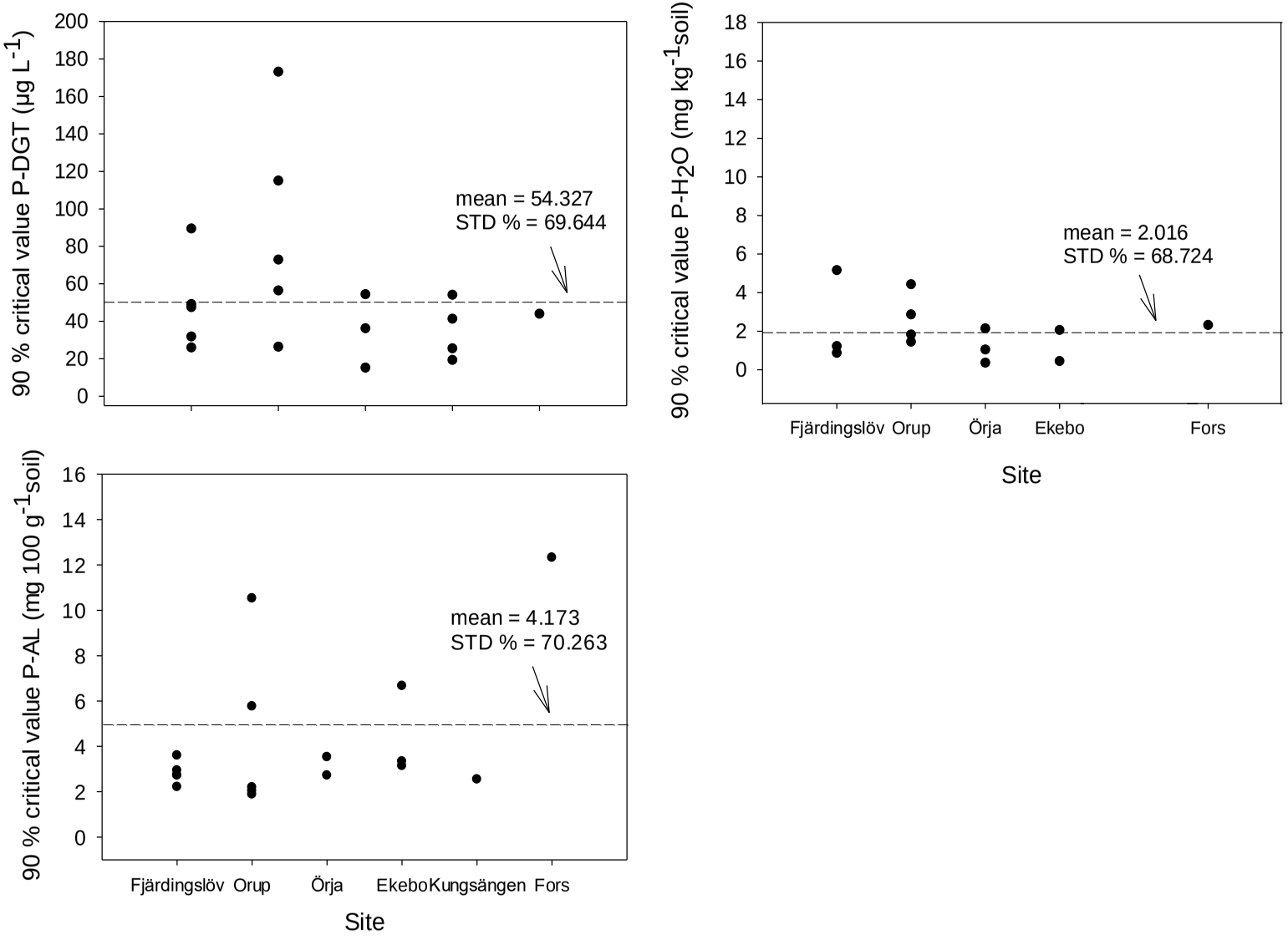


Fig. 16: 90 % critical values over all years split by site. Dashed line represents annotated mean value across sites.

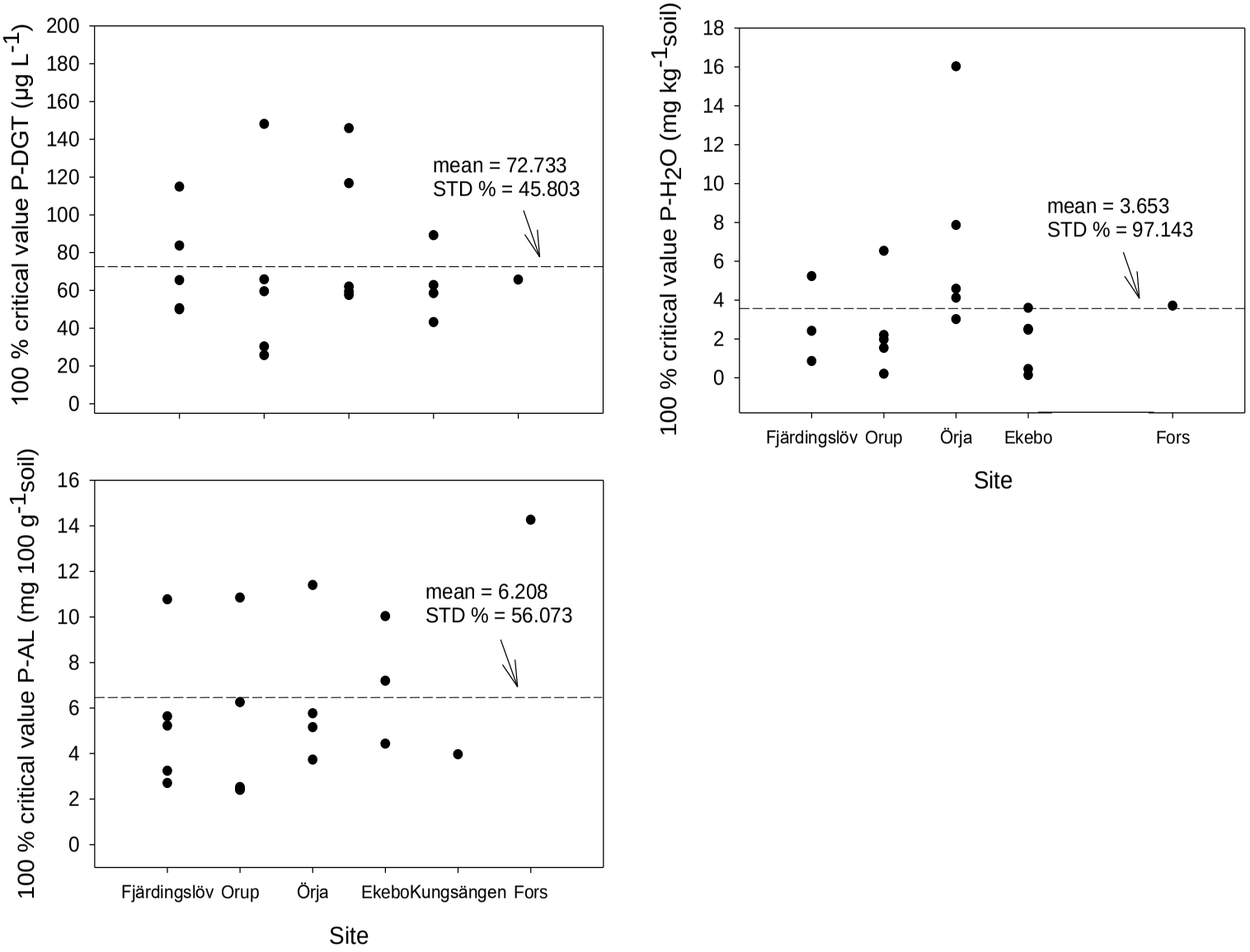


Fig. 17: 100 % critical values over all years split by site. Dashed line represents annotated mean value across sites.

Table 16: Comparison of 90 % critical yield thresholds of P-DGT and P-AL for different years calculated with the piecewise global curve fit and the Mitscherlich model.

Method	Year	90 % threshold piecewise global curve fit	90 % threshold Mitscherlich model
P-DGT (µg L^{-1})	1967	52.66	34.70
	1967-1979	41.48	27.35
	1967-1991	41.07	24.87
	2003-2015	75.16	40.55
	1967-2015	54.33	27.04
P-AL (mg P 100 g^{-1} soil)	1967	3.81	2.02
	1967-1979	4.34	2.01
	1967-1991	4.32	1.60
	2003-2015	3.97	2.21
	1967-2015	4.87	2.11

Comparison of the 90 % critical thresholds of P-DGT and P-AL using the different curve fits, shows large differences (Table 16). Thresholds calculated with the pairwise global curve fit are twice as high as the thresholds calculated using the Mitscherlich model. The trends of critical thresholds of different combinations of years are however similar for both models.

3.4 Treatment and P-DGT change

Figure 18 shows the yearly P-DGT change with yearly applied P fertilizer the dashed line indicating zero change. Below a P application of 17 kg P ha^{-1} $year^{-1}$ P-DGT decreases yearly by up to -5 % for a 0 P treatment. The yearly P-DGT change is smaller between the 0P and the replacement P (15) treatment and higher (steeper slope) between the replacement (15) and the replacement + 30 kg P ha^{-1} $year^{-1}$ (45) fertilization. When the critical P-DGT value, meaning the value above which relative yield is bigger than 90 %, is achieved, this level can be maintained by fertilizing yearly more than 17 kg P ha^{-1}.

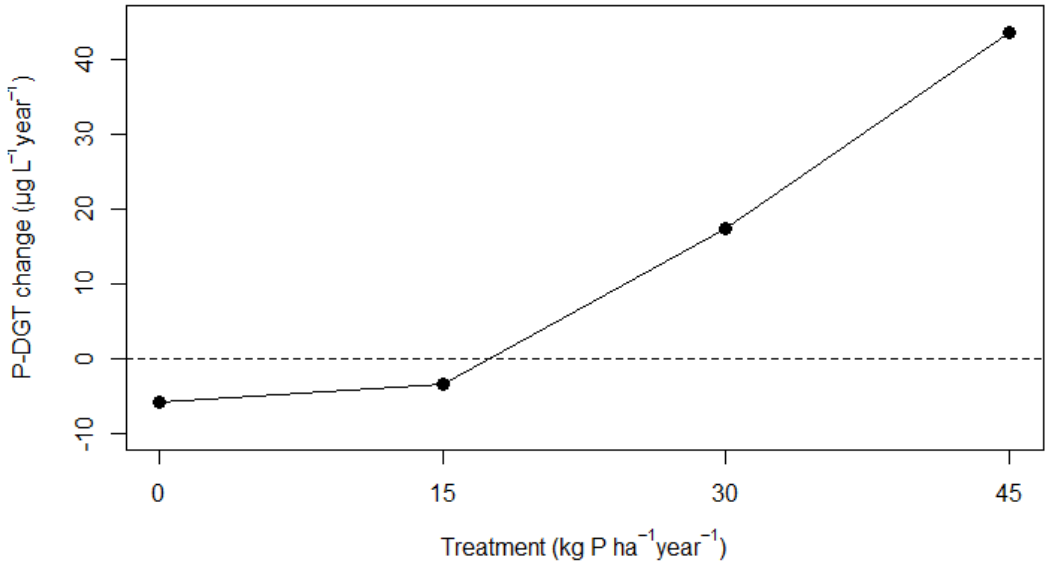


Fig. 18: Relationship between yearly applied P and yearly P-DGT change calculated across all sites and years. Dashed line represents zero line to maintain the current P-DGT value.

3.5 Correlations between extracted P data and other soil properties

The overall correlation of pH and P-DGT was rather poor (R = 0.172, Table 17). However, when splitting sites and years, differences of correlations were found. While the two favorable sites Fjärdingslöv and Örja showed no correlation of pH with P-DGT, correlations at Orup and Ekebo were moderate (R = 0.392 and 0.400). Furthermore, correlations of pH and P-DGT at Orup and Ekebo in the years 1979 and 2003 were high (> 0.5). Overall, correlations varied a lot over the years at the same site.

Table 17: Pearson correlation between pH and P-DGT, pH and % relative yield.

Site	Year	pH – P-DGT
All	All years	0.172
Fjärdingslöv	All years	-0.462
Orup	All years	0.392
Örja	All years	-0.042
Ekebo	All years	0.400
Orup	1967	0.274
	1979	0.761
	1991	0.378
	2003	**0.903**
	2015	0.090
Ekebo	1967	0.458
	1979	0.550
	1991	-0.393
	2003	**0.830**
	2015	0.362

3.6 Swiss samples

As the number of treatments was limited at the Swiss site, one soil and three P-levels, data evaluation was less useful. Pearson correlation coefficients between the extracted P of the different soil-P tests and grain yield were low and showed no improvement when correlating extracted P with early dry yield, although it is assumed that most P is taken up in early stages of plant growth (Grant et al. 2001), (Table 18). Moreover, P contents of plant biomass related poorly with extracted P. However, highest correlations were found between the three P tests and plant P contents. Extracted P of the three extraction methods DGT, water and AL showed strong positive relationships (Fig. 19, 20, 21).

Table 18: Pearson correlation coefficients between extracted P of the three different soil-P test and different plant and soil factors

Factor	P-DGT	P-H_2O	P-AL
Grain yield	0.25	0.21	0.18
Early grain yield	-0.16	-0.22	-0.15
Plant-P	0.44	0.53	0.6
pH	0.35	0.32	0.33

3.6.1 Internal relationship between soil P extraction methods

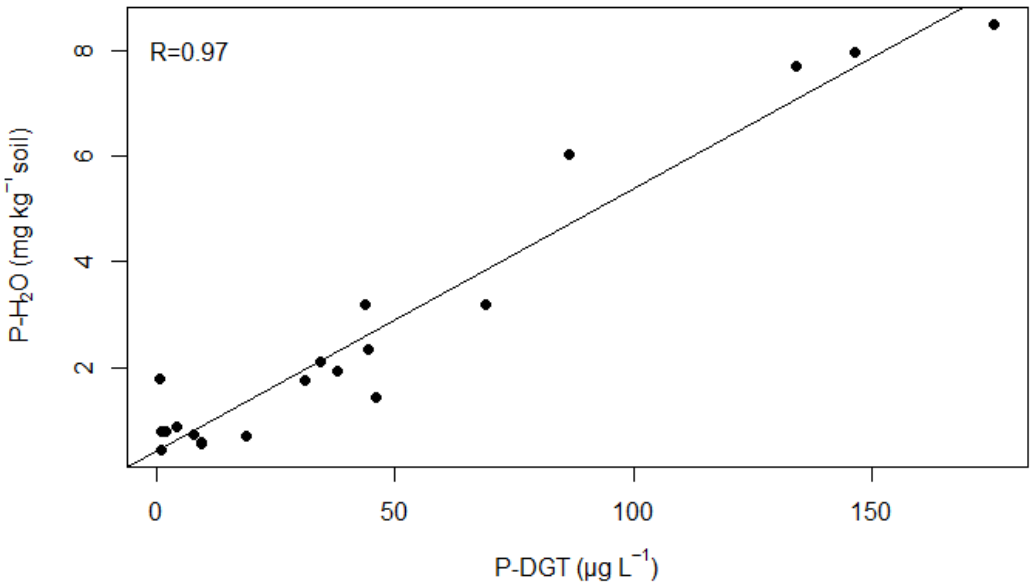


Fig. 19: Relationship between soil extracted P using DGT and water extraction. The R value in the upper left corner indicates the Pearson correlation coefficient.

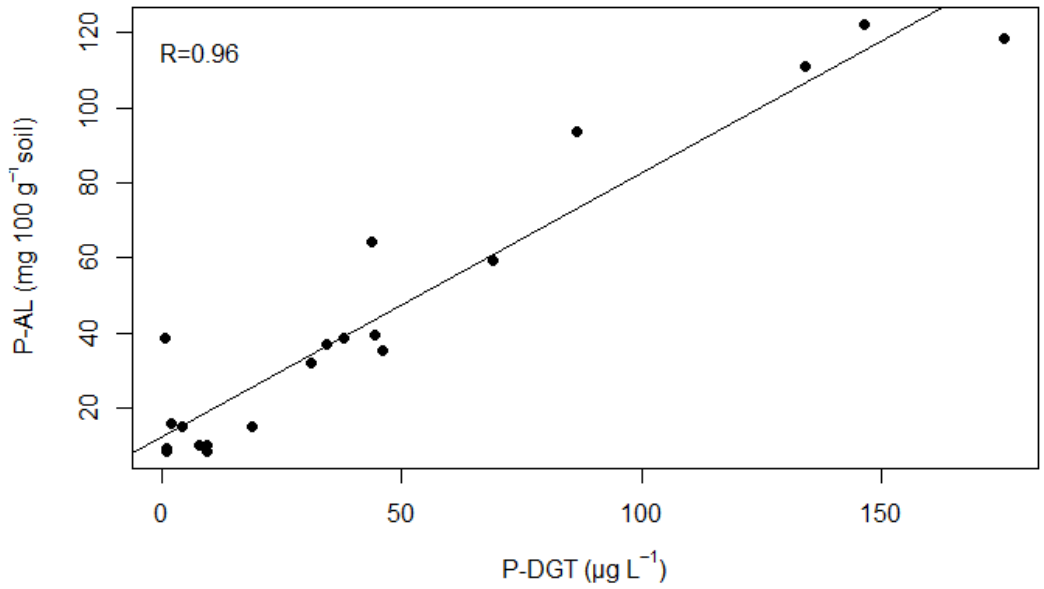


Fig. 20: Relationship between soil extracted P using DGT and AL. The R value in the upper left corner indicates the Pearson correlation coefficient.

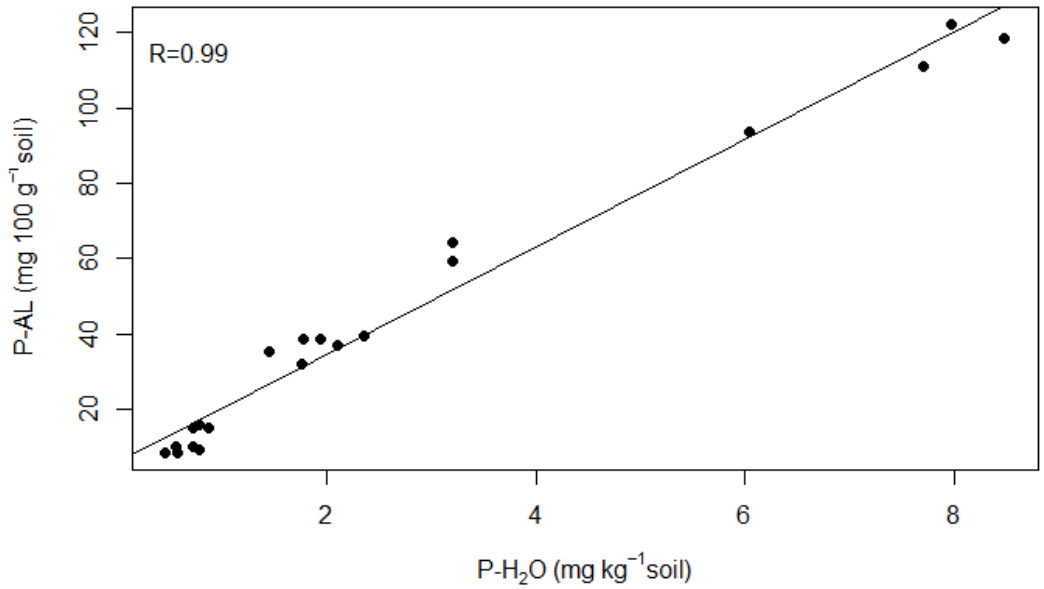


Fig. 21: Relationship between soil extracted P using water extraction and AL. The R value in the upper left corner indicates the Pearson correlation coefficient.

4 Discussion

4.1 Yield response to applied P

Mean grain yields across all years for the four sites varied between 4364 to 5458 kg ha^{-1}, caused by a combination of environmental, climate and soil conditions at every site (Table 7). Fjärdingslöv and Örja have favorable soil conditions, which are mainly due to the parent rock and high clay contents (Ivarsson & Bjarnason 1988) and mean yields are highest at those sites (5382 and 5458 kg dry matter ha^{-1}, respectively). Already in 1967, ten years after the field experiment was launched, grain yields of Fjärdingslöv and Örja were around 1000 kg dry matter ha^{-1} or 25 % higher than at Orup and Ekebo (Fig. 7). Switching to higher yielding wheat varieties caused an increasing yield trend observed since 1979 until 2015.

One aspect of grain yield fluctuations might be spatially uneven distribution of fertilizer due to their granular nature and strong bounding of P to soil surfaces. Several studies report that solid fertilizers can cause point effects on plant yield (Dilz and van Brakel 1985). Compared to the 0 P rate, all other P fertilization rates increased yields significantly (Fig. 6 and Table 6). However, grain yields decreased for the treatments with higher P rates (replacement +15 kg P ha^{-1} and replacement +30 kg P ha^{-1}). Particularly in 1991, grain yields seem to be unrelated to fertilizer P rates. Pot trials with controlled conditions usually show clear yield responses to fertilizer P, such as Mason et al. (2010) discovered in greenhouse experiments with wheat or Six et al. (2014) with maize. In comparison, field experiments do not allow to keep environmental factors stable and could therefore result in more variable yields independently of fertilized P. However, to my knowledge, there are no studies reporting decreasing yields with increasing P fertilizer and no explanations could be found for the decreasing yields in the present study. Thus, the observed yield penalty beyond the replacement P treatment indicated that replacement P seems to be the optimum amount of applied P fertilizer and P was not a limiting factor for grain yield beyond more application than replacement.

Additionally, physical soil properties such as the soil texture, soil aeration and biological properties such as microbial activity and the presence of mycorrhiza could enhance or decrease plant available P. Also micronutrients such as copper, manganese and zinc could have influenced grain yields (Syers et al. 2008). Hamnér (2016) pointed out in her doctoral thesis that micronutrient concentrations in Swedish soils are estimated to be in the low or average range, as the parent geologic material granite and gneiss are low in micronutrients and humid conditions lead to leaching of these elements.

4.1.1 Mitscherlich model

In this study, P input and grain yield were not always positively correlated (mainly for the two upper P inputs) and simply using relative yields was not possible. After excluding sites that were not responsive at all (Table 8, last column "excluded"), the commonly used Mitscherlich model was used to fit responses between predicted maximum grain yield and P rate for 16 out of 19 sites (Table 8). Coefficients of determination were high ($R^2 \geq 0.8$), which is in accordance with Mason et al. (2010) who also found high R^2 values for 19 out of 22 sites when fitting

maximum wheat yield to fertilizer P addition. Only two sites showed a linear relationship during certain years (Table 8). Thus, for most years the Mitscherlich model was adequate to predict yield response to fertilized P.

4.2 Comparison of soil P extracted by different methods

Extracted P-DGT from soil ranged from 2.24 to 457.30 µg L^{-1} (Table 9) and is thus similar to data obtained from Mason et al. (2010), Mason et al. (2013) and McBeath et al. (2007) using Australian agricultural soils. As values obtained with the DGT method do not refer to soil mass, numbers are not directly comparable with P results of other extractable P methods. Water extracted P amounted to values of 0.13 to 17.16 mg P kg^{-1} soil (Table 9) and were relatively low compared to the mean value of 18 mg P kg^{-1} soil of 12 European countries (Schick et al. 2013). In contrast, values for extracted P-AL ranged between 110 and 2460 mg P kg^{-1} soil (Table 10) and appeared to be higher compared to the mean value of 1590 mg P kg^{-1} soil of 12 European countries (Schick et al. 2013).

For the P 0 treatment, plant-available P measured with DGT accounted for about 20 µg P L^{-1} in 2015. In the other treatments, values of P-DGT were higher and thus increased over time (Fig. 11). Furthermore, P-DGT differences between sites became larger with increased fertilized P indicating that site-specific properties influence the amount and speed of P plant availability change. Fjärdingslöv and Örja exhibited overall the highest plant available phosphorus measured with the DGT method suggesting that soil conditions at those two sites promoted P plant availability.

Higher concentrations of P-AL compared to P-H_2O corresponded with data of Schick et al. (2013), Neyroud & Lischer (2003) and Wuenscher et al. (2015) who also found water extracting the least P. Compared to the acidic AL method applied in Sweden, water extraction is representing mobile P only and was shown to relate better to crop response than the P-AL method (Van der Paauw et al. 1971). Generally, acidic extractions such as AL or alkaline methods extract also non-plant available P making the extraction method soil-type dependent. In contrast, water alters the P solubility at a minimum maintaining the pH within one unit of the original soil pH (Self-Davis et al. 2000). Thus, water soluble P represents the actual solubility of soil phosphorus and water extraction may serve as a complement or even alternative to AL or other chemical extraction methods. Moreover, water extraction provides information about the fate of phosphorus running off or being leached from agricultural fields and is thus of environmental interest (Moore et al. 1998). Additionally, the low costs and time spent on the measurement procedure, as well as the significant correlations between water extraction and other methods, make water extraction a suitable routine analysis (Fischer et al. 2017).

When extractable P derived by different methods was related to rates of P fertilizer applied across sites and years, highest extracted P values represented the highest P rate, the second highest extracted P value represented the second highest P rate etc. (Fig. 9). Thus, extracted P data distinguished between different P rates. Fjärdingslöv and Örja exhibited the highest

extracted P values which is in accordance with Ivarsson & Bjarnason (1988) describing these two sites as favorable and fertile. However, soil properties that could influence the amount of plant available P measured by DGT do not clearly explain the higher P-DGT values at the two sites. While pH values at Örja (favorable) and Ekebo (not favorable) were within the optimum range of 6-7 for plant-available P, Fjärdingslöv (favorable) and Orup (not favorable) showed alkaline and acidic pH values (7.8 and 5.3) promoting fixation of phosphorus as Ca-phosphates and Al-phosphates, respectively (Silva, 2012) (Table 3). As Ca-phosphates are considered not to be plant available, P-DGT values at Fjärdingslöv would be expected to be lower than at Örja and Ekebo. Furthermore, the content of organic carbon in the soil, whose positive effect on P plant availability was confirmed by several authors (e.g., Syers et al. 2008; Fink et al. 2016), was higher at Orup and Ekebo, although these sites showed lower P-DGT values. Factors that could promote plant-available P at Fjärdingslöv are a higher percentage of total P and a higher percentage of vermiculites compared to the other sites. Vermiculite consists of stable Al polymers within interlayer spaces. The alkaline pH at Fjärdingslöv can decrease the reactivity of Al by forming stable interlayer Al polymers leading to a considerable reduction of the specific surface of Al and P adsorption sites (Hall and Baker 1971). Thus, the chance of phosphorus getting bound to Al was lower at this site.

At all sites and for all methods, extracted P showed depletion for the 0 P treatment and stayed at the same level for the 15 kg P replacement treatment (Fig. 9, 10, 18). This trend was also reported in previous studies of the Swedish soil fertility experiments (Table 19). This is in accordance with studies from Rubaek & Sibbesen (2000), Rodríguez (2016) and Zhan et al. (2015) reporting that P was depleted over time without P addition. On average, extracted P values for the two high P rates in the present study (30 and 45 kg P ha^{-1} $year^{-1}$) increased over the years, which was also observed by Rubaek & Sibbesen (2000), Rodríguez (2016) and Zhan et al. (2015) who found rising Olsen-P/Bondorff P (for 30 kg P ha^{-1} $year^{-1}$), rising extractable P measured with the Chang and Jackson fractionation method (for 32 and 67 kg P ha^{-1} $year^{-1}$) and Olsen-P (for 33 and 65 kg P ha^{-1} $year^{-1}$). For the two upper P rates at the sites Fjärdingslöv, Orup and Örja in the present study, P-DGT and P-H_2O showed the same trend with slightly decreasing values until 1991 followed by a peak in 2003 and decreasing values in 2015. No reasonable explanation was found for the peak in 2003. Conjectures of measurement errors are not likely as extracted P peaks in 2003 were systematical and peak values occurred for the same homogenized soil samples after analysis using DGT and water extraction.

In contrast to the initially decreasing and later increasing trend of P-DGT and P-H_2O for the two high P application rates, P-AL tended to increase slightly over the years. The similarity of P-DGT and P-H_2O trends, which was not observed for P-AL, indicated that the two methods using water extracted similar P pools. DGT uses passive diffusion to an infinite sink, while during the water extraction, phosphorus is solved by shaking of the soil solution whereby water acted as a sink. Both methods use water as an extractant but the ratio of soil to water is different with a ratio of 1 to 10 for water extraction and around 1 to 0.5 for the saturation paste (DGT). Several studies have shown strong covariations between DGT and soil solution concentrations. The DGT method can also serve as dynamic speciation tool providing information about dissociation kinetics of complexes in solution by using the ratio of cDGT to total solution

concentration (Degryse et al. 2009). This explains the similarity of extracted P by DGT and water. However, since AL applies an acidic ammonium acetate lactate extraction, different pools are solved and released than with water. At Ekebo, all three soil tests showed the same trend for extracted P.

Table 19: Mean change of P-AL of 10 Swedish soils including Fjärdingslöv, Örja and Ekebo with and without applied animal manure (Bergström et al. 2015).

Type of P addition	Total P applied	Rate of P-AL† change
P-depleting treatments	kg P ha^{-1} yr^{-1}	mg P 100 g^{-1} soil yr^{-1}
No P	0	−0.062
Manure P only (5 t wet weight yr^{-1})	7.5	−0.049
Replacement of P removed by crops	15	−0.032
P-enriching treatments		
Replacement + 15 kg fertilizer P	30	0.055
Replacement + 20 kg fertilizer P	35	0.086
Replacement + 30 kg fertilizer P	45	0.157

Overall, values for extracted P from the different methods showed good correlations (Table 11) and P-DGT and P-H_2O correlated with 0.874 the most, which corresponds to the review by Degryse et al. (2009) who reported DGT and soil solution concentrations having co-varied well in several studies. P-AL and P-H_2O also exhibited a good regression coefficient of 0.703, which is slightly weaker than the relationships found by Schick et al. (2013) who examined 217 soil samples from across Europe and obtained an R^2 of 0.907 between both methods. Although several authors such as Neyroud & Lischer (2003) and Jordan et al. (2012) claimed that extracted P values of different standard soil tests correlate well, discussions about the comparability of soil tests are controversial. Jordan et al. (2012) criticized firstly the widely differing amount of extracted P and secondly the restricted applicability to all soil types, e.g., AL was reported to overestimate plant available P of alkaline soils. Neyroud & Lischer (2003) even received differing P results using the same extraction method, but analyzed in different laboratories. Furthermore, the scientific basis for using specific extractants is often poor and soil tests are simply chosen as standard national soil test for practical reasons (Jordan et al. 2012).

Although extractable P tests are generally well correlated, at the Fors site no correlations between P-DGT and P-AL and P-H_2O and P-AL (R^2 = -0.226 and 0.015) were found. Fors is classified as Calcaric Paeozem in the FAO system and is characterized by a high $CaCO_3$ content of 3.5 % in the topsoil layer and a pH of 7.3. Investigating 82 Swedish topsoil samples, Otabbong et al. (2009) showed that the strong acidic ammonium lactate dissolves insoluble Ca-P compounds and hydrolyzes P of insoluble Al-humic-P substances. Thus, plant available P of alkaline soils is often overestimated by the AL method. Similarly, results of Hill (2016) indicated carbonate content having an influence on extracted P of conventional methods, while DGT performed more conformal across 4 Austrian sites with different $CaCO_3$ contents. The high carbonate content at Fors compared to the other sites is therefore likely to be the cause for the weak relationships of extractable soil P methods in the present study. However, P-DGT and

P-H_2O correlate relatively well, as both use water as an extractant that is not influenced by a high carbonate content.
In the present study, only the effect of pH and carbonate content were examined and other possibly influencing soil properties such as the type of clay mineral or organic P were considered, though not entirely investigated. Wuenscher et al. (2015) found soil properties influencing extractable P differently when using a number of extraction methods. Thus, further research is needed to test the varying influences of soil characteristics on different tests; especially as the DGT method holds promise to be rather independent of soil properties.

4.3 Yield response to soil P data

When comparing grain yields over the years and extracted P trends over the years, none of the soil-tests showed similar trends (Fig. 7, 9 and 10). While P-DGT and P-H_2O showed a drop in 1991, grain yields dropped in 1979 followed by an increase over the years until 2015. Furthermore, both DGT and water extraction showed the lowest mean value of extracted P at Ekebo accompanied by the second lowest yield at this site (Table 9 and 10). In contrast, P-AL was highest at Ekebo (Table 10). Thus, DGT and water extraction seem to provide more reliable results than AL as an indicator. Generally, it has to be kept in mind, that plant-available P showed variations year by year, which is due to the uneven spreading of fertilizer granules and differing soil sampling spots (Dilz and van Brakel 1985; Carlgren & Mattsson 2001). In the present study, soil samples from only every twelfth year were used, hence annual variations are not available.

4.3.1 Mitscherlich model

One possible way to establish a relationship between crop yield and soil P data was to fit data to the Mitscherlich model. Values R^2 predicting yield response to P-DGT were throughout lower than for the piece-wise data fit for different combinations of years (R^2 = 0.36 to 0.64, Fig. 12 and Table 16). The more years were included in the model calculation, the lower became the R^2 value and the accuracy of the model declined. However, when looking at one year (here 1967) the Mitscherlich curve fit produced a coefficient of determination of 0.64, which is similar to the value obtained by Mason et al. (2010) for selected sites (R^2 = 0.74). In their study, yield responses to fertilized P could be explained by the Mitscherlich model at 20 sites but failed at 15 sites. The inclusion of all sites resulted in a considerably weaker correlation (R^2 = 0.44). In the present study a relatively small data set was used, only four sites could be included, and low to moderate R^2 values (R^2 = 0.36 to 0.64) that are similar to the R^2 value of Mason et al. (2010) were obtained. Yields in the present study represented both spatial and temporal data across 48 years. Most likely, the Mitscherlich model may not be superior over the piecewise regression fit to assess critical P values for maximum yield.

Extracted P-AL showed weaker correlations to maximum relative yield than P-DGT (Fig. 13 and Table 12). Coefficients of correlation were below 0.40 for all years except for the years 2003 and 2015, where AL performed better than DGT (0.77 compared to 0.44). The sharply rising P-DGT in 2003 not measured with P-AL was not reflected in higher relative yields. Still, the Mitscherlich model provided better yield response for P-DGT than for P-AL.

4.3.2 Piecewise linear regression

The other method applied to gain critical P threshold values for maximum yield was the piecewise linear regressions showing high coefficients of determination between extracted P and relative yield (Table 14). The highest R^2 values were obtained for three out of four soils using P-DGT. Generally, yields increase with nutrient addition until a certain threshold where the nutrient is not limiting anymore before flattening out to a plateau. Contradictive to this general expected yield response behavior, yields in this study increased with extracted P until a certain threshold but were often followed by a slight decrease beyond the threshold (Fig. 14 and 15). This suggested that P was not the limiting factor above a certain level but other factors adversely influenced or limited the yield. Piecewise curve fits between yield and extractable P in soil revealed a relatively steep increase, i.e., a narrow range of extracted P values before the critical threshold for maximum yield was achieved especially for water and AL (Fig. 15). Duboc et al. (2017) also found a narrow range of water extracted P from fertilizers.

Critical thresholds for soil P to achieve 90 % relative yield were determined manually from the regression plots. These data were compared with the 90 % critical thresholds derived with the Mitscherlich model. However, values for the 90 % critical threshold deviated largely from the 100 % critical threshold as yield responses were steep (Fig. 16 and 17). The similarity of yield response for P-DGT and P-H_2O was probably due to the use of water as the same extractant and covariations between soil solution concentrations and DGT that have been reported by Degryse et al. (2009). With DGT critical threshold values ranging between 35.058 and 88.792 µg P L^{-1}, these are similar to other studies. Mason et al. (2010) determined a 90 % critical threshold of 66 µg L^{-1} for wheat growing in Australia, while Six et al. (2014) and Six et al. (2012) obtained 51 and 73 µg L^{-1} to achieve 80 % yield.

Standard deviations of soil P data to achieve maximum yield between sites indicated the accuracy of the methods. With relative standard deviations decreasing in the order P-DGT < P-H_2O < P-AL and highest coefficients of determination obtained between extracted P-DGT and relative yield, the DGT technique seems to be the most accurate method to estimate critical thresholds across different sites (Table 14 and Fig. 16 and 17). This is in accordance with other studies (Table 1). Menzies et al. (2005) reported a strong relationship between P-DGT, soil solution P and tomato yield in pot experiments as compared to the Bray and Colwell method. McBeath et al. (2007) investigated 28 Australian soils in a greenhouse with spring wheat and found high correlations to P-DGT for liquid and granular fertilizer (R^2 = 0.82 and 0.74, respectively). Moreover, Six et al. (2012, 2014) found stronger relationships between relative yield and P-DGT than for other methods in pot experiments. Other studies also showed strong relationships between yields and DGT compared to other extractable soil P tests (Table 1). However, larger deviations are to be expected when field trials are used instead of pots (Fageria 2017; Poorter et al. 2012)

Until now, only two articles have been published investigating the relationship between extracted P-DGT and yield under field conditions. Mason et al. (2010) compared wheat response to fertilized P of 35 different sites in southern Australia and fitted a Mitscherlich model describing the relationship between P-DGT and relative yield. DGT could explain 74 % of the

grain yield variation compared to 35 % using the P-resin method. For the Colwell soil test, no significant relationship between extracted P and relative yield were found. The majority of studies listed in Table 1 proofed the strength of DGT compared to other, particularly chemical soil-P methods, to predict yield. In contrast, Mundus et al. (2017) reported results investigating 34 P responsive soils from Scandinavia in a greenhouse experiment. In their study, neither leaf tissue P concentrations nor dry matter of spring barley correlated with P-DGT or other P extraction methods. Furthermore, they carried out a field experiment with nine P responsive soils cultivating spring barley and after 30 days, all methods applied correlated well with P concentrations in the youngest fully emerged leaf with DGT performing best ($R^2 = 0.83$). After further three weeks, extractable soil P methods were not able to predict relative yield or leaf P concentrations anymore. However, Mundus et al. (2017) mentioned the unusually cold weather during the spring barley growing season as a possible reason for weak correlations. Similarly, Hill (2016) investigating four Austrian sites under field conditions found no statistically significant relationships between P extracted by DGT, CAL or EDTA and relative yield. In an investigation using 164 South Australian soil samples from 1968 to 2008, (Speirs et al. 2013) related P extracted by different methods with relative yields. Slightly lower R^2 values for P-DGT than Colwell P with smaller confidence intervals were found. These results suggest that field conditions have a greater impact on plant yields and that P might not have been the sole or main limiting factor.

4.3.3 Comparison of the piecewise linear regression and the Mitscherlich model

Comparing the 90 % critical thresholds of P-DGT determined by the piecewise curve fit with the values from the Mitscherlich model showed, on average, 76 % higher thresholds for the piecewise linear regression (Table 16). However, when combining years, similar thresholds were derived, the lowest for the years 1967-1991 and the highest for 2003 and 2015. Apparently, the two curve fits showed the same trend over years, but on a different level.

The Mitscherlich model was first tested per site and year relating the relative yield to applied P; the piecewise regression was first tested to fit data per site and year relating the relative yield to P-DGT. However, already at this point, R^2 values for the piecewise linear regression were slightly higher and only one site in one year needed to be excluded compared to four sites to be excluded using the Mitscherlich model. Additionally, critical thresholds derived from piecewise fits were determined per site and year and then averaged to critical thresholds per site. In contrast, critical thresholds of the Mitscherlich model were only calculated for different combinations of years but not per site. As the Mitscherlich model was fitted by Mason (2017) and the piecewise regression fit was applied subsequently and by myself, fitting procedures differed and are thus not directly comparable.

Most likely, the Mitscherlich model may not be superior over the piecewise regression fit to assess critical P values for maximum yield.

4.3.4 Usefulness of soil P extraction for fertilizer P requirement

The critical threshold for a certain soil represents the level of available P above which the relative yield is 90 % (Holford et al. 1985). On average at all sites, critical thresholds for the

different P extraction methods were reached with P fertilizer applied equivalent to replacement. Below the critical value, loss of yield can be expected, while P application above the critical value does not result in higher yields but generates more costs for farmers and possibly lead to eutrophication (Schröder et al. 2010). At the critical level, readily available soil P is used effectively and replaced with P fertilizer (Syers et al. 2008). Once the critical threshold has been reached in the Swedish soil experiments, P addition of 17 kg P ha^{-1} $year^{-1}$ would on average maintain the plant-available P, while less fertilized P would lead to P deficiency (Fig. 18). The same value of 17 kg P ha^{-1} $year^{-1}$ was found by Bergström et al. (2015) at which AL-extracted P remained constant. Furthermore, depletion of extractable soil P is slower than enrichment as can be seen from the steepness of the slopes. As seen, P-DGT can be a very useful tool to more accurately detect critical thresholds in soil.

4.3.5 Site-specific soil properties and limitations of soil P extraction methods

Besides soil properties that might affect plant-available P in the topsoil, other site-specific soil characteristics shown in Table 3, including deeper horizons, might influence the actual P uptake and thus the plant yield. Both soils, Fjärdingslöv and Ekebo, exhibit a high bulk density in the subsoil, while Fjärdingslöv has a higher bulk density in the topsoil (1.70 kg dm^{-3} until 40 cm) compared to Ekebo (1.52 kg dm^{-3}). At first sight, this seems to be contradictory to the higher yields at Fjärdingslöv, as a higher bulk density leads to lower porosity and thus aggravates root extension (Syers et al. 2008). However, field studies showed that root penetration into the subsoil is more restricted at Ekebo than at Fjärdingslöv. This might be a consequence of smectites found in the lowest horizon at Fjärdingslöv (0.80 – 1 m) whose shrinking and swelling processes lead to cracks in the soil allowing roots to penetrate the relatively dense soil (Kirchmann & Eriksson 1993). Furthermore, the higher share of calcium carbonate at Fjärdingslöv favors soil aggregate structure. At Orup, root growth is assumed to be limited by the high bulk density in the subsoil and potentially plant available water would only last for 17 days during a dry period (Kirchmann & Eriksson 1993). On average, yields at Örja were 1 t ha^{-1} yr^{-1} higher than at Orup, which is owed to the high porosity of the subsoil, the relatively large quantity of plant available water and the optimal pH at Örja.

The goal of the different P extraction methods to measure the amount of phosphorus available for crop uptake seems fairly utopic. Although the DGT method uses undisturbed soil samples and the close-to-reality diffusion process as a measure of plant availability, other soil, plant physiological or environmental factors influence the actual P uptake by plants which no extraction method including DGT can account for. Root system size of plants and its structure are genetically determined but are also influenced by soil properties such as pH, bulk density or plant available water (Syers et al. 2008). Particularly the fine root hairs and the 1-2 mm space around roots, the so-called rhizosphere, play a major role in plant nutrient availability (Jungk 2001). Additionally, microbial activity in the rhizosphere increases the P availability by decreasing the pH and solubilizing Al- and Fe-phosphates in the rhizosphere and organic P is mineralized by microorganisms to plant-available forms. Furthermore, organic acids excreted by roots can solubilize mineral P (Mikkelsen 2013).

Besides the plant physiology, laboratory conditions differ from natural field conditions. While the soil is wetted in the laboratory to 85 % of its maximum water holding capacity (DGT) or mixed with an access of solutes (chemical extractions), soil moisture in the field is usually lower or shows seasonal and yearly variations. Additionally, the empirically determined DGT deployment time of 24 hours does not reflect the time period of phosphorus uptake from plants, which can be much longer (Syers et al. 2008). Thus, only potentially plant available P data can be gained with any extraction method.

4.4 Swiss samples

Results from the Swiss experiment indicated weak relationships between soil P extracted by DGT, AL and water with plant yield or pH (Table 18). However, stronger relations of extracted P with plant P contents with R^2 values of 0.44 to 0.6 were found. Still, these are slightly lower than data reported by Tandy (2011), $R^2 = 0.72$, from greenhouse experiments. Correlations between the three soil P extraction methods were high, 0.96, 0.97 and 0.99 (Fig. 19, 20, 21), indicating a similar performance. However, limited data did not allow further analyses.

5 Conclusion

The Diffusive Gradient in Thin Film (DGT) method seems to be superior over the water and ammonium-acetate-lactate extraction to estimate relative wheat yields and critical thresholds of different sites by determining soil extractable P if P is diffusion limited. However, the DGT method is still relatively costly and time consuming. The piecewise linear regression is likely to be more suitable than the Mitscherlich model to establish a relationship between crop yield and soil extractable P data and to determine extractable P required for maximum wheat yield. Multiple factors such as environment, climate or plant physiological properties influence the actual plant P uptake and consequently yields, which no extraction method including DGT can account for. Thus, only potentially plant-available P data can be gained with any extraction method. The DGT method can be a very useful tool to more accurately detect critical thresholds in soil and derive adequate P fertilizer amounts; hence the DGT method should be made accessible for farmers around the world.

6 References

Alexandersson, H., Karlström, C., & Larsson-McCann, S. (1991). Temperature and precipitation in Sweden 1961-90. Reference normals. Sveriges Meteorologiska och Hydrologiska Inst.

Barrow, N.J. & Shaw, T.C., 1976. SODIUM BICARBONATE AS AN EXTRACTANT FOR SOIL PHOSPHATE, I. SEPARATION OF THE FACTORS AFFECTING THE AMOUNT OF PHOSPHATE DISPLACED FROM SOIL FROM THOSE AFFECTING SECONDARY ADSORPTION. *Geoderma*, 16, pp.91–107.

Bergström, L. et al., 2015. Turnover and Losses of Phosphorus in Swedish Agricultural Soils: Long-Term Changes, Leaching Trends, and Mitigation Measures. *Journal of Environmental Quality*, 44, pp.512–523.

Bray, R.H. & Kurtz, L.T., 1945. Determination of total, organic, and available forms of phosphorus in soils. *Soil Science*, 59, pp.39–45.

Burkitt, L.L. et al., 2016. The ability of the DGT soil phosphorus test to predict pasture response in Australian The ability of the DGT soil phosphorus test to predict pasture response in Australian pasture soils – a preliminary assessment. *Soil Use and Management*, 32(March), pp.27–35.

Carlgren, K. & Mattsson, L., 2001. Swedish Soil Fertility Experiments. , 51(September), pp.49–76.

Davison, B. et al., *DGT – for measurements in waters , soils and sediments*, Available at: www.dgtresearch.com.

Davison, W. & Zhang, H., 1994. in situ speciation measurements of trace components in natural water using thin-film gels. *Nature*, 367, pp.546–548.

Degryse, F. et al., 2009. Predicting availability of mineral elements to plants with the DGT technique: A review of experimental data and interpretation by modelling. *Environmental Chemistry*, 6(3), pp.198–218.

Demaria, P. et al., 2005. Exchangeability of phosphate extracted by four chemical methods §. *Plant Nutrition Soil Science*, 168, pp.1–5.

Demetz, M. & Insam, H., 1999. Phosphorus availability in a forest soil determined with a respiratory assay compared to chemical methods. *Geoderma*, 89, pp.259–271.

Duboc, O. et al., 2017. Predicting phosphorus availability from chemically diverse conventional and recycling fertilizers. *Science of the Total Environment*, 599–600, pp.1160–1170. Available at: http://dx.doi.org/10.1016/j.scitotenv.2017.05.054.

Egner, H., Riehm, H. & Domingo, W.R., 1960. Untersuchungen über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nährstoffzustandes von Böden II. Chemische Extraktionsmetoden zur Phosphor- und Kaliumbestimmung. *Kungliga Lantburkshögskolands annaler*, 26, pp.199–215.

Eriksson, A.K., 2009. Phosphorus in agricultural soils around the Baltic Sea - Comparisons of different laboratory methods as indices for phosphorus leaching to waters. , (64).

Fageria, N.K., 2017. Soil Fertility and Plant Nutrition Research Under Controlled Conditions : Basic Principles and Methodology Soil Fertility and Plant Nutrition Research Under Controlled Conditions : Basic Principles. *Journal of Plant Nutrition*, 28, pp.1975–1999.

FAO, 2015. *World fertilizer trends and outlook to 2018*,

Gallet, A. et al., 2003. Effect of phosphate fertilization on crop yield and soil phosphorus status. *Journal of Plant Nutrition and Soil Science*, 166(5), pp. 568-578.

Grant, C.A. et al., 2001. Importance of Early Season Phosphorus Nutrition. *Better Crops*, 85(2), pp.18–23.

Hamel, C. & Strullu, D., 2006. Arbuscular mycorrhizal fungi in field crop production : Potential and new direction. *Canadian Journal of Plant Science*, 86(4), pp.941–950.

Hamnér, K., 2016. *Micronutrients in Cereal Crops Impact of Nutrient Management and Soil Properties*. Swedish University of Agricultural Sciences Uppsala.

Heidari, S. et al., 2016. A New Method of Preparing Gel for DGT Technique and Application to the Soil Phosphorus Availability Test. *Communications in Soil Science and Plant Analysis*, 47(10), pp.1239–1251. Available at: http://www.tandfonline.com/doi/full/10.1080/00103624.2016.1166248.

Hill, B., 2016. Diffusive Gradients in Thin Films (DGT): a novel technique to predict plant response to nutrient availability.

Holford, I.C.R. et al., 1985. Yield Responsiveness and Response Curvature as Essential Criteria for the Evaluation and Calibration of Soil Phosphate Tests for Wheat. *Australian Journal of Soil Research*, 23, pp.167–180.

Hooda, P.S. et al., 1999. Measuring bioavailable trace metals by diffusive gradients in thin ® lms (DGT): soil moisture effects on its performance in soils. *European Journal fo Soil Science*, 50(June), pp.285–294.

Jasinski, S., 2017. *Phosphate rock*,

Jordan, L. et al., 2012. An overview of fertilizer-P recommendations in Europe : soil testing , calibration and fertilizer recommendations. *Soil Use and Management*, 28, pp.419–435.

Jungk, A., 2001. Root hairs and the acquisition of plant nutrients from soil. *Journal of Plant Nutrition and Soil Science*, 164(2), pp.121–129. Available at: http://doi.wiley.com/10.1002/1522-

2624(200104)164:2%3C121::AID-JPLN121%3E3.3.CO;2-Y.
Kirchmann, H., 1991. Properties and Classification of Soils of the Swedish Long-term Fertility Experiments Properties and Classification of Soils of the. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science*, 41, pp.227–242.
Kirchmann, H. & Eriksson, J., 1993. Properties and Classification of Soils of the Swidish Long-Term Fertility Experiments. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science*, 43:4(January 2016), pp.193–205.
Kirchmann, H., Eriksson, J. & Snäll, S., 1999. Swedish Long-Term Fertility Experiments : IV . Sites at Ekebo and Fjärdingslöv Properties and Classification of Soils of the Swedish Long-Term Fertility Experiments. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science*, 49, pp.25–38.
Knight, B. & Mcgrath, S., 1998. In Situ Measurements of Solution Concentrations and Fluxes of Trace Metals in Soils Using DGT. *Environmental Science and Technology*, 32, pp.704–710.
MacDonald, G.K. et al., 2011. Agronomic phosphorus imbalances across the world's croplands. *Proceedings of the National Academy of Sciences of the United States of America*, 108(7), pp.3086–91. Available at: http://www.scopus.com/inward/record.url?eid=2-s2.0-79952607805&partnerID=tZOtx3y1.
Mason, S., Hamon, R., et al., 2008. Investigating chemical constraints to the measurement of phosphorus in soils using diffusive gradients in thin films (DGT) and resin methods. *Talanta*, 74, pp.779–787.
Mason, S., Mcneill, A. & Mclaughlin, M.J., 2010. Prediction of wheat response to an application of phosphorus under field conditions using diffusive gradients in thin-films (DGT) and extraction methods. *Plant Soil*, 337, pp.243–258.
Mason, S., Mcneill, A. & Sites, F., 2008. Diffusive Gradients in Thin-films (DGT) as a technique for accurately predicting Phosphorus fertiliser requirements DGT (C E) % relative P responsive Crop type µ g / L. *Soil & Land Systems University of Adelaide*, (September).
Mason, S.D. et al., 2013. Soil test measures of available P (Colwell , resin and DGT) compared with plant P uptake using isotope dilution. *Plant Soil*, 373(1), pp.711–722.
McBeath, T.M. et al., 2007. Predicting the response of wheat (Triticum aestivum L .) to liquid and granular phosphorus fertilisers in Australian soils. *Australian Journal of Soil Research*, 45, pp.448–458.
Menzies, N.W., Kusumo, B. & Moody, P.W., 2005. Assessment of P availability in heavily fertilized soils using the diffusive gradient in thin films (DGT) technique. *Plant and Soil*, 269, pp.1–9.
Mikkelsen, R., 2013. A Closer Look at Phosphorus Uptake by Plants. *IPNI Insights*, (Juli), pp.1–4.
Mundus, S., Carstensen, A. & Husted, S., 2017. Predicting phosphorus availability to spring barley (Hordeum vulgare) in agricultural soils of Scandinavia. *Field Crops Research*, 212(July), pp.1–10. Available at: http://dx.doi.org/10.1016/j.fcr.2017.06.026.
Nawara, S. et al., 2017. A comparison of soil tests for available phosphorus in long-term field experiments in Europe. *European Journal of Soil Science*, (January), pp.1–13. Available at: http://doi.wiley.com/10.1111/ejss.12486.
Neyroud, J. & Lischer, P., 2003. Do different methods used to estimate soil phosphorus availability across Europe give comparable results ? *Journal of Plant Nutrition and Soil Science*, 166, pp.422–431.
Otabbong, E. et al., 2009. Compatibility of the ammonium lactate (AL) and sodium bicarbonate (Olsen) methods for determining available phosphorus in Swedish soils. *Soil and Plant Science*, 59(March 2017), pp.373–378.
Poorter, H. et al., 2012. The art of growing plants for experimental purposes: a practical guide for the plant biologist. *Functional Plant Biology*, 39, pp.821–838.
Portch, S. & Stauffer, M.D., 2005. Soil testing: a proven diagnostic tool. *Better Crops*, 89(1), pp.28–32.
Rodríguez, I.G., 2016. *Fate of phosphorus in soil during a long term fertilization experiment in southern Finland*. University of Helsinki.
Rubaek, G.H. & Sibbesen, E., 2000. Long-term phosphorus fertilization - Effects on crop yield and soil phosphorus status. *DIAS*, 31(July).
Santner, J. et al., 2010. Ferrihydrite containing gel for chemical imaging of labile phosphate species in sediments and soils using diffusive gradients in thin films. *Analytical Chemistry*, 82(18), pp.7668–7674.
Scally, S., Davison, W. & Zhang, H.A.O., 2003. In Situ Measurements of Dissociation Kinetics and Labilities of Metal Complexes in Solution Using DGT. *Environmental Science and Technology*, 37, pp.1379–1384.
Schick, B.J. et al., 2013. *Comparison and Inter-Calibration of Different Soil P Tests Used in the Baltic Sea Countries Comparison and Inter-Calibration of Different Soil P Tests Used in the Baltic Sea Countries*,
Schröder, J.J. et al., 2010. Sustainable use of phosphorus. *Plant Research International Wageningen*, 357, pp.1–124. Available at: http://www.susana.org/docs_ccbk/susana_download/2-1587-sustainableuseofphosphorusfinalsustpenvb120090025.pdf.
S. Sinaj et al., 2009. Données de base pour la fumure des grandes cultures et des herbages (DBF-GCH) *Rev. Suisse Agric.*, 41, pp. 1-98.
Six, L., Pypers, P. & Degryse, F., 2012. The performance of DGT versus conventional soil phosphorus tests in tropical soils - An isotope dilution study. *Plant Soil*, 359, pp.267–279.

Six, L., Smolders, E. & Merckx, R., 2014. Testing phosphorus availability for maize with DGT in weathered soils amended with organic materials. *Plant Soil*, 376, pp.177–192.

Six, L., Smolders, E. & Merckx, R., 2012. The performance of DGT versus conventional soil phosphorus tests in tropical soils — maize and rice responses to P application. *Plant Soil*, 359.

Speirs, S., Scott, B. & Moody, P.W., 2013. Soil phosphorus tests II : A comparison of soil test – crop response relationships for different soil tests and wheat. *Crop and Pasture Science*, 64(November), pp.469–479.

Speirs, S.D. et al., 2013. Soil phosphorus tests II : A comparison of soil test – crop response relationships for different soil tests and wheat Soil phosphorus tests II : A comparison of soil test – crop response relationships for different soil tests and wheat. *Crop and Pasture Science*, 64(5), pp.469–479.

Syers, J.K., Johnston, A.E. & Curtin, D., 2008. Efficiency of soil and fertilizer phosphorus use. *FAO fertilizer and plant nutrition bulletin*, 18.

Tandy, S. et al., 2011. The use of DGT for prediction of plant available copper, zinc and phosphorus in agricultural soils. *Plant and Soil*, 346(1), pp.167–180.

Wuenscher, R. et al., 2015. A comparison of 14 soil phosphorus extraction methods applied to 50 agricultural soils from Central Europe. *Plant Soil Environment*, 61(2), pp.86–96.

Zhan, X. et al., 2015. Changes in Olsen Phosphorus Concentration and Its Response to Phosphorus Balance in Black Soils under Different Long-Term Fertilization Patterns. *PLOS ONE*, pp.1–15.

Zhang, H. et al., 1998. In situ measurement of dissolved phosphorus in natural waters using DGT. *Analytica Chimica Acta*, 370, pp.29–38.

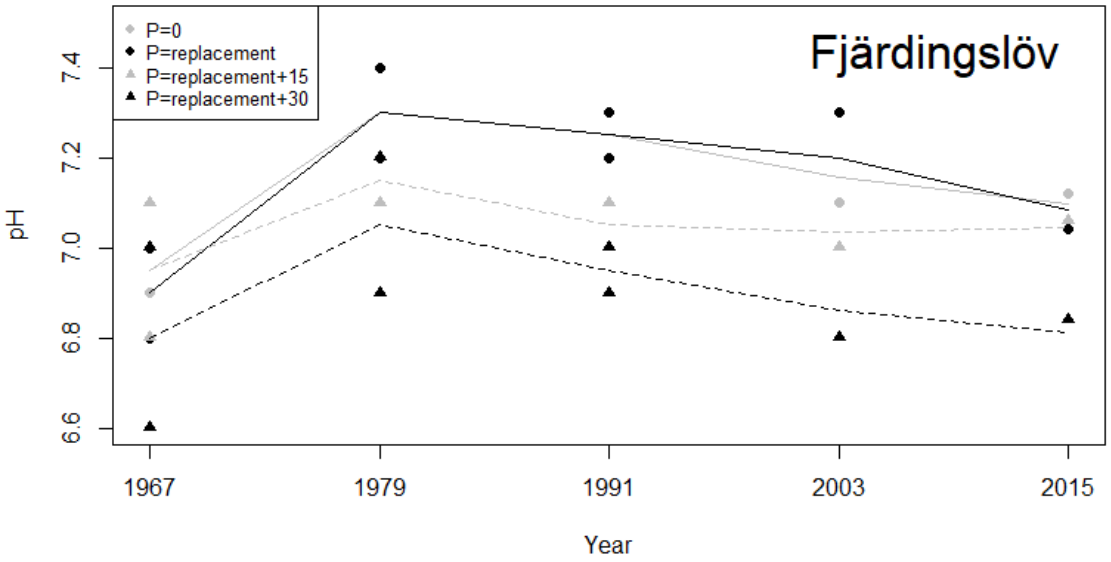
Zhang, H. & Davison, W., 1995. Performance Characteristics of Diffusion Gradients in Thin Films for the in Situ Measurement of Trace Metals in Aqueous Solution. *Analytical Chemistry*, 67(19), pp.3391–3400.

Zhang, Y. et al., 2013. Optimization of the diffusive gradients in thin fi lms (DGT) method for simultaneous assay of potassium and plant-available phosphorus in soils. *Talanta*, 113, pp.123–129.

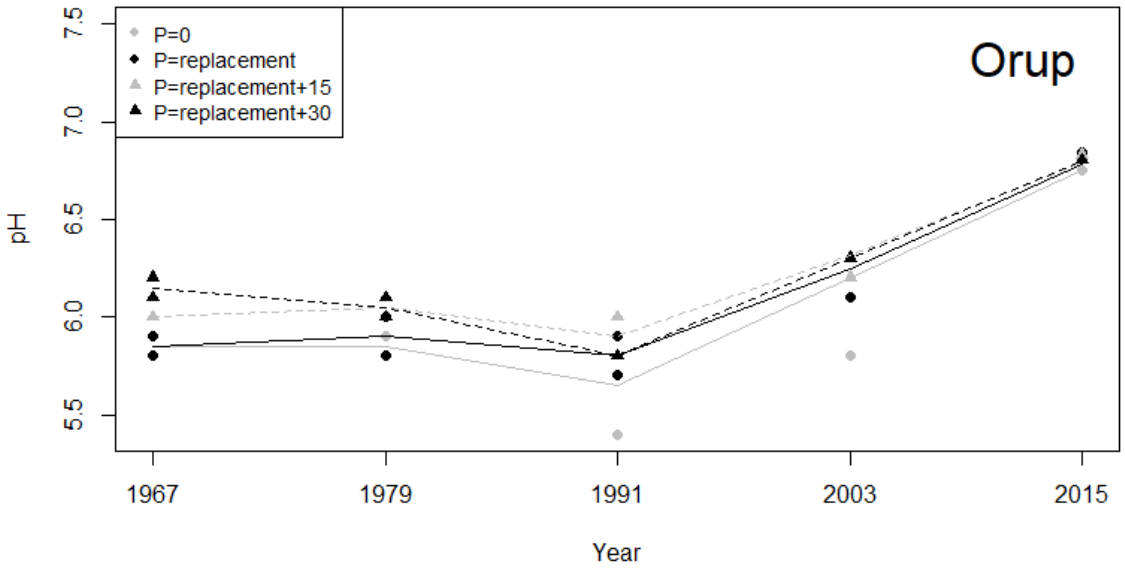
Acknowledgments

This study was carried out as a cooperation between the University of Natural Resources and Life Sciences Vienna, Austria and the University of Agricultural Sciences Uppsala, Sweden. Special thanks go to my first supervisor Holger Kirchmann for his time and effort and whose challenging questions always led to vivid discussions. I sincerely thank my co-supervisor Walter Wenzel I developed the study design with and who guided me with his valuable ideas throughout the project. Thanks to Sokrat Sinaj for the provision of soil samples and some thoughts. I am truly grateful to Olivier Duboc for his constant support and instructions in the laboratory. I want to thank Markus Puschenreiter, Veronika Slavik and Jakob Santner for their availability and answering of questions concerning laboratory work. Thanks to John Eric Florida and Christina Hipfinger for having helped me with soil sample measurements. I would also like to thank Gunnar Björson for having provided soil samples. Lastly, I would like to thank my family for their encouragement and trust in me.

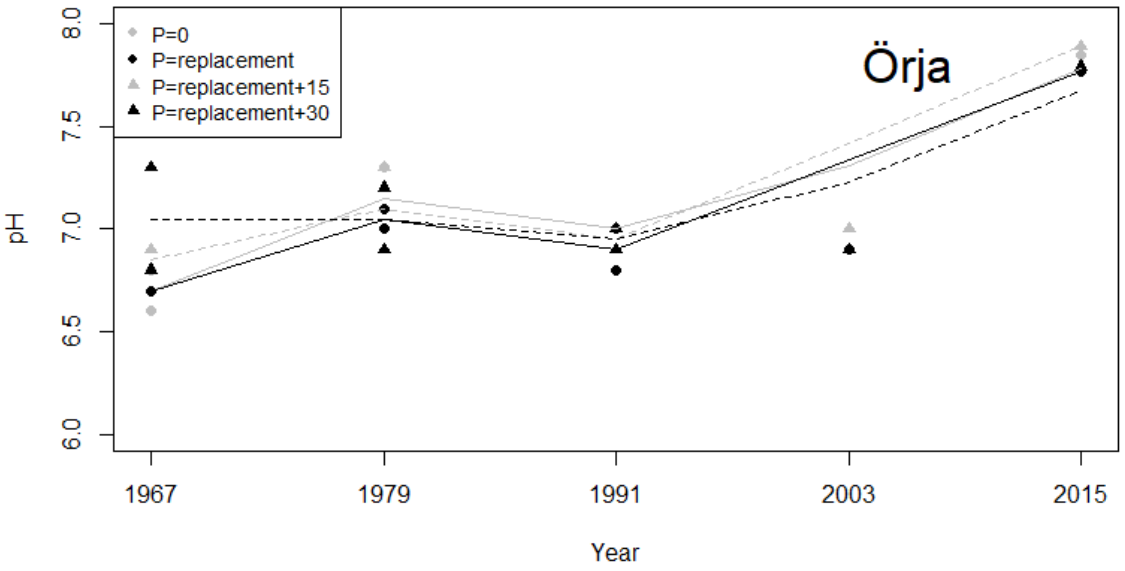
7 Appendix



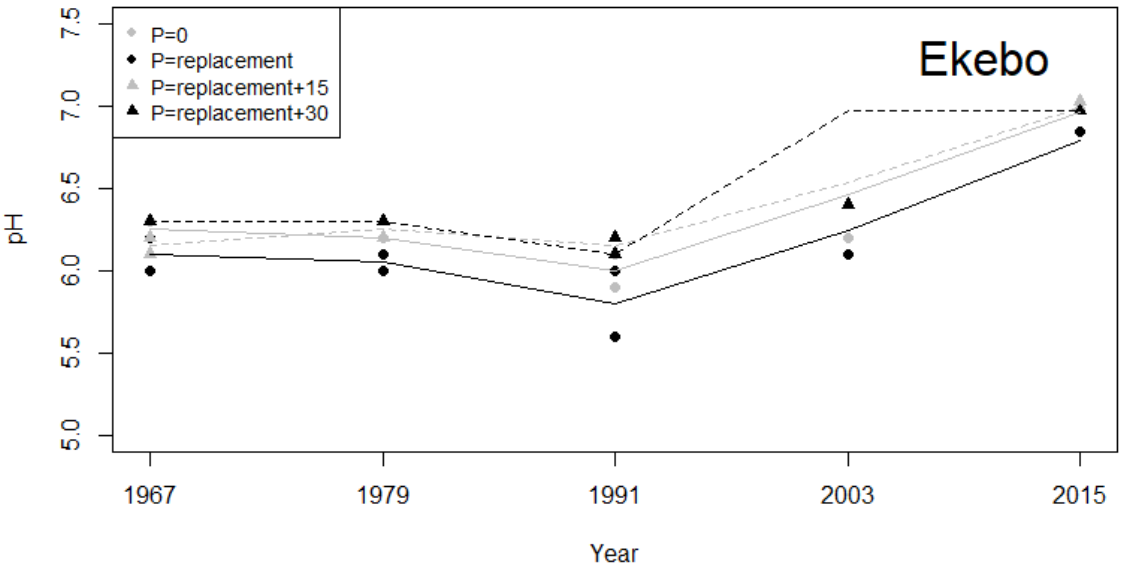
Appendix figure 1: pH values over years at Fjärdingslöv.



Appendix figure 3: pH values over years at Örja.



Appendix figure 2: pH values over years at Orup.



Appendix figure 4: pH values over years at Ekebo.

Appendix table 1: Extracted P measured with the DGT, AL and water extraction method for all sites and years.

Year	Site	Treatment (kg ha^{-1} y^{-1})	P-DGT (µg L^{-1})	P-H_2O (mg P kg^{-1} soil)	P-Al (mg P 100 g^{-1} soil)	Grain yield (kg dry matter ha^{-1})	Rel. Yield (%)
1967	Fjärdingslöv	0	31.50	0.63	2.70	3646.50	70.07
1967	Fjärdingslöv	15	50.50	1.12	3.90	4889.50	93.93
1967	Fjärdingslöv	30	85.50	3.83	4.75	4748.50	91.23
1967	Fjärdingslöv	45	142.00	8.60	8.10	4878.50	93.73
1967	Orup	0	28.00	1.01	1.20	3404.00	79.41
1967	Orup	15	54.00	1.83	2.05	3858.50	90.00
1967	Orup	30	52.50	2.87	3.35	4058.50	94.67
1967	Orup	45	85.50	4.95	5.20	4050.00	94.47
1967	Örja	0	41.50	0.97	2.90	5322.00	95.53
1967	Örja	15	62.00	3.68	3.95	5498.50	98.70
1967	Örja	30	120.50	3.89	6.50	5337.00	95.79
1967	Örja	45	196.00	9.23	8.25	5266.00	94.53
1967	Ekebo	0	44.50	1.56	4.35	4651.00	87.43
1967	Ekebo	15	41.00	1.37	5.45	4434.00	83.36
1967	Ekebo	30	62.50	2.50	7.20	4960.00	93.24
1967	Ekebo	45	90.00	3.90	9.05	4553.50	85.60
1967	Kungsängen	0	65.00	1.26	3.90	5482.50	86.67
1967	Kungsängen	30	50.00	1.26	4.20	5969.00	94.36
1967	Kungsängen	45	54.50	1.00	3.90	5923.50	93.64
1967	Fors	0	145.00	4.16	10.40	5763.50	93.77
1967	Fors	30	106.50	2.31	9.75	6090.50	99.09
1967	Fors	45	115.50	2.94	10.35	5811.00	94.55
1979	Fjärdingslöv	0	17.00	0.68	1.95	4075.50	86.37
1979	Fjärdingslöv	15	36.50	1.85	4.05	4443.00	94.16
1979	Fjärdingslöv	30	94.00	3.50	7.40	4505.50	95.48
1979	Fjärdingslöv	45	178.50	7.06	12.95	4140.50	87.75
1979	Orup	0	22.50	0.94	1.20	3355.50	79.96
1979	Orup	15	33.50	1.99	2.40	4173.00	99.43
1979	Orup	30	61.00	2.65	4.60	3758.50	89.55
1979	Orup	45	91.50	5.60	8.30	3462.00	82.50
1979	Örja	0	21.50	0.81	1.95	4127.50	84.95
1979	Örja	15	38.00	2.29	3.70	4402.50	90.62
1979	Örja	30	94.00	4.92	8.25	4667.50	96.06
1979	Örja	45	138.50	6.17	13.15	4549.00	93.63
1979	Ekebo	0	10.50	1.53	3.60	2797.50	64.00
1979	Ekebo	15	20.50	1.17	6.25	3165.50	72.41
1979	Ekebo	30	48.50	3.54	10.10	3918.00	89.62
1979	Ekebo	45	60.50	4.49	13.75	3735.00	85.44
1979	Kungsängen	0	41.00	0.67	2.40	4672.50	88.64
1979	Kungsängen	15	57.50	0.61	3.25	5067.50	96.13
1979	Kungsängen	30	95.50	2.00	6.15	5172.00	98.12
1979	Kungsängen	45	116.00	2.01	6.75	5109.50	96.94

Year	Site	Treatment (kg ha^{-1} y^{-1})	P-DGT (µg L^{-1})	P-H_2O (mg P kg^{-1} soil)	P-Al (mg P 100 g^{-1} soil)	Grain yield (kg dry matter ha^{-1})	Rel. Yield (%)
1979	Fors	0	30.00	2.04	10.90	4014.50	77.23
1979	Fors	15	21.50	2.08	13.45	5198.00	100.00
1979	Fors	30	51.00	3.06	15.15	5054.50	97.23
1979	Fors	45	69.50	2.49	15.85	4642.50	89.31
1991	Fjärdingslöv	0	24.00	0.33	1.60	5457.00	89.52
1991	Fjärdingslöv	15	48.50	1.31	6.10	5552.50	91.09
1991	Fjärdingslöv	30	126.00	1.37	7.60	4888.00	80.18
1991	Fjärdingslöv	45	143.00	5.23	14.70	4682.50	76.82
1991	Orup	0	17.00	0.40	1.20	4911.50	83.28
1991	Orup	15	32.00	1.53	2.65	4816.00	80.81
1991	Orup	30	60.00	2.23	5.10	5303.50	89.92
1991	Orup	45	106.00	3.10	9.95	5345.00	90.63
1991	Örja	0	16.50	0.36	1.95	5996.50	90.02
1991	Örja	15	57.50	1.37	3.90	6327.00	95.05
1991	Örja	30	108.50	3.90	9.05	5939.50	89.16
1991	Örja	45	149.50	3.83	14.75	6015.00	90.29
1991	Ekebo	0	27.50	0.62	2.40	5354.50	96.97
1991	Ekebo	15	22.00	1.05	5.30	4822.00	87.33
1991	Ekebo	30	69.00	3.64	9.85	3334.50	60.39
1991	Ekebo	45	106.00	4.75	13.70	3593.00	65.07
2003	Fjärdingslöv	0	31.00	0.86	1.30	5955.00	76.68
2003	Fjärdingslöv	15	109.00	2.99	4.40	7346.00	94.59
2003	Fjärdingslöv	30	240.50	9.67	9.90	7766.00	100.00
2003	Fjärdingslöv	45	159.00	17.16	16.50	7561.00	97.37
2003	Orup	0	43.50	0.24	1.50	3792.00	68.82
2003	Orup	15	136.00	1.76	2.40	5308.00	96.32
2003	Orup	30	254.50	10.43	6.40	5510.00	100.00
2003	Orup	45	160.00	14.67	11.90	5507.00	99.93
2003	Örja	0	23.50	0.26	2.30	6223.00	87.32
2003	Örja	15	147.00	3.36	4.00	6988.00	98.05
2003	Örja	30	358.00	11.97	14.00	7127.00	100.00
2003	Örja	45	235.50	16.12	16.10	7037.00	98.73
2003	Ekebo	0	16.00	0.45	2.70	5195.00	86.38
2003	Ekebo	15	45.00	0.73	6.50	5865.00	97.52
2003	Ekebo	30	109.50	3.94	11.40	5762.00	95.81
2003	Ekebo	45	78.50	7.42	18.10	6014.00	100.00
2015	Fjärdingslöv	0	20.00	0.14	1.35	6909.00	81.73
2015	Fjärdingslöv	15	100.00	1.30	3.78	8453.00	100.00
2015	Fjärdingslöv	30	258.50	6.10	10.54	8201.00	97.03
2015	Fjärdingslöv	45	174.50	12.38	17.57	8219.00	97.24

Year	Site	Treatment (kg ha^{-1} y^{-1})	P-DGT (µg L^{-1})	P-H_2O (mg P kg^{-1} soil)	P-Al (mg P 100 g^{-1} soil)	Grain yield (kg dry matter ha^{-1})	Rel. Yield (%)
2015	Orup	0	7.50	0.13	2.00	4839.00	97.55
2015	Orup	15	59.50	0.20	4.10	4960.00	100.00
2015	Orup	30	202.00	4.07	10.30	4443.00	89.56
2015	Orup	45	156.00	5.42	16.80	4395.00	88.59
2015	Örja	0	22.00	0.31	1.90	7658.00	94.91
2015	Örja	15	61.50	0.53	3.60	7863.00	97.46
2015	Örja	30	170.00	3.15	6.00	7673.00	95.10
2015	Örja	45	126.00	7.86	11.40	8068.00	100.00
2015	Ekebo	0	13.50	0.14	2.60	7540.00	88.70
2015	Ekebo	15	63.00	0.84	7.50	8500.00	100.00
2015	Ekebo	30	160.00	4.11	18.10	8299.00	97.63
2015	Ekebo	45	216.00	8.19	24.60	7737.00	91.01